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2001-45028 26 July 2001 (26.07.2001) KR(71) Applicant (for all designated States except US): MEDEX-
GEN CO. LTD. [KR/KR]; 2th Floor, Medical Bldg A,
Hanyang University College of Medicine, 17 Haengdang-
dong, Seongdong-gu, 133-791 Seoul (KR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): CHUNG,

Yong-Hoon [KR/KR]; #405-804 Jugong Apt., Dun-
chon-dong, Gandong-gu, 134-060 Seoul (KR). HAN,
Ji-Woong [KR/KR]; #201 HanYang Villa 24-5,
Guseui-2dong, Gwangjin-gu, 143-816 Seoul (KR).
LEE, Hye-Ja [KR/KR]; #607 ChungSil Apt., Gae-
honghon-dong, Guro-gu, 152-806 Seoul (KR). CHOI,
Eun-Yong [KR/KR]; 19-1 Chungchun-1dong, Pupy-
ong-gu, 403-854 Incheon-si (KR). KIM, Jin-Mi [KR/KR];
409-287 Shillimbondong, Gwanak-gu, 151-029 Seoul
(KR).

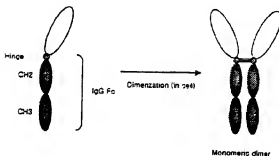
(74) Agents: LEE, Sei-Jin et al.; 17th Floor, City Air Tower,
159-9 Samsung-dong, Gangnam-gu, 135-973 Seoul (KR).

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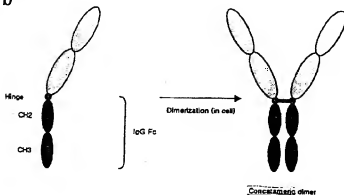
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(54) Title: CONCATAMERIC IMMUNOADHESION

a



b



(57) Abstract: Disclosed are concatameric proteins comprising two soluble domains, in which the C-terminus of a soluble domain of a biologically active protein is linked to the N-terminus of an identical soluble domain or a distinct soluble domain of a biologically active protein. Also, the present invention disclosed dimeric proteins formed by formation of intermolecular disulfide bonds at the hinge region of two monomeric proteins formed by linkage of a concatamer of two identical soluble extracellular regions of proteins involving immune response to an Fc fragment of an immunoglobulin molecule, their glycosylated proteins. DNA constructs encoding the monomeric proteins, recombinant expression plasmids containing the DNA constructs, host cells transformed or transfected with the recombinant expression plasmids, and a method of preparing the dimeric proteins by culturing the host cells. Further, the present invention disclosed pharmaceutical or diagnostic compositions comprising the dimeric protein or its glycosylated form.

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CONCATAMERIC IMMUNOADHESION

TECHNICAL FIELD

5 The present invention relates to concatameric proteins, and more specifically, concatamerized structure of biologically active protein domains where C-terminal end of extracellular soluble domain of biologically active protein is fused to N-terminal end of the same or other extracellular soluble domain of biologically active protein, and dimerization of two concatamers by coupling to hinge region of Fc fragment of immunoglobulin, and glycosylated forms of the concatameric proteins.

BACKGROUND ART

10 The activity of cytokine is associated with pathologic severity of inflammatory and /or immune response to various antigenic stimulations. Many antigen specific antibodies and soluble receptors which could recognize cytokines are currently in use to inhibit the function of cytokines for the therapeutic purposes (WO 93/016184, WO 96/02576, WO 96/023067, WO 1997/03682, and US 5,434,131, 5,656,272, 5,977,318, 6,210,661, 6,225,117). Antibodies and soluble receptors inhibit cytokine signal transduction by disturbing interaction between cytokines and their receptors on cell surface.

20 Soluble receptors used as functional inhibitors of cytokine that fused to heavy chains of human immunoglobulins were disclosed by Capon et al. (Nature 337:5254, 1989), and thereafter many patents were disclosed inventions related to fusion proteins of soluble receptors and immunoglobulins (US patent 5,521,288, 5,844,095, 6,046,310, 6,090,914, 6,100,383, 6,225,448).

Generally, fusion proteins of soluble receptors and immunoglobulins have following advantages (Capon et al., Nature 337:5254, 1989)

1. Increase in total avidity to ligand by forming bivalency via dimerization.
2. Increase in blood half-life of proteins, that is, increase in molecular stability
3. Activation of effector cells by Fc fragment of immunoglobulin heavy chain
4. Convenience of purification by using affinity column, e.g. using protein A

Most fusion proteins of receptor extracellular domain and immunoglobulin heavy chain are composed of heavy chain without CH1 domain, which result in dimers not binding to light chains. This structure is more desirable for the function of proteins and receptors involving immune response. For example, TNFR(WO92/16221, WO95/34326)-immunoglobulin fusion proteins disclosed in WO94/06476 and US 5,447,851 have been used for the inhibition of TNF-mediated inflammation. It is well known that TNFR-immunoglobulin fusion proteins have a higher affinity than original monomeric molecules (Lesslauer et al., Eur. J. Immunol. 21:2883, 1991; Ashkenazi et al., Proc. Natl. Acad. Sci. 88:10535, 1991; Peppe et al., J. Exp. Med. 174:1483, 1991; Mohler et al., J. Immunol. 151:1548, 1993).

For the improved inhibition of TNF mediated response, one can increase efficacy by multimerizing soluble extracellular domains of TNFR, CD2, and CTLA-4. For example, when fusion proteins of TNFR's extracellular domains bound with immunoglobulin heavy chain (heavy chain fusion protein) and with light chain (light chain fusion protein) respectively are coexpressed in the same cell, one can produce fusion proteins as a tetrameric form by linking heavy chain to heavy and light chains. This tetramer showed much more increased efficacy than monomeric or dimeric forms as presented by Scallon et al. (Cytokine 7:759, 1995).

However, this method had many difficulties for commercialization such as simultaneous expression of two different fusion genes in the same cell line, remarkably lower production yields of multimeric form; and difficulty in purifying multimeric high

molecular weight forms. For these reasons, immunoglobulin fusion proteins currently in use are only heavy chain fused form.

Therefore, there is considerable demand for the development of methods of producing multimeric protein therapeutics with high yield and efficient purification procedures.

DISCLOSURE OF INVENTION

5 The present inventors have manufactured concatameric proteins by fusing the C-terminal end of soluble domain of biologically active protein to the N-terminal end of soluble domain of the same or other biologically active protein by using DNA recombination techniques. Also, the present inventors have dimerized this concatamers by linking it to the hinge region of Fc fragment of immunoglobulin and added more
10 glycosylations by using DNA mutagenesis techniques. And the present inventors have found that concatamerized protein dimers and their glycosylated forms show increased efficacy and stability compared to conventional monomeric fusion proteins.

 Therefore, in one aspect, the present invention provides concatameric proteins where C-terminal end of soluble domain of biologically active proteins is fused to N-
15 terminal end of soluble domain of the same or other biologically active proteins.

 In another aspect, the present invention provides dimeric proteins formed by disulfide bond at hinge region of two monomeric proteins whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulin.

 Also in another aspect, the present invention provides DNA constructs that
20 encode monomeric fusion proteins whose concatamerized domain is fused to hinge region of Fc fragment of immunoglobulins.

Also in another aspect, the present invention provides DNA plasmids comprising a DNA construct that encodes monomeric fusion protein whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulin.

Also in another aspect, the present invention provides host cells transfected or transformed with recombinant DNA plasmids including a DNA construct that encodes monomeric fusion protein whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulin.

Also in another aspect, the present invention provides a method for culturing the host cells, which were transfected or transformed with recombinant DNA plasmids including a DNA construct that encodes monomeric fusion protein whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulin, under culture condition for expression of DNA constructs encoding concatameric fusion protein coupled to hinge region of Fc fragment of immunoglobulin, and manufacturing dimeric concatamers formed by disulfide bond at hinge region of two monomeric concatamers described as above including the process of purification of the proteins described as above from cell culture.

Also in another aspect, the present invention provides a method for culturing the host cells, which were transfected or transformed with recombinant DNA plasmids including a DNA construct that encodes monomeric fusion protein whose concatamerized part of immunomodulatory function is fused to hinge region of Fc fragment of immunoglobulin and is inserted with glycosylation motifs, under the best condition which is suitable for expression of DNA constructs that encode monomeric fusion protein whose concatamerized part of immune function is fused to hinge region of Fc fragment of immunoglobulin, and for manufacturing glycosylated dimers formed by disulfide bond at hinge region of two monomeric proteins described as above including the process of purification of the glycosylated proteins described as above from cell culture.

Also in another aspect, the present invention provides DNA primers for inserting glycosylation motif into the DNA constructs that encode monomeric fusion

proteins whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulins.

Also in another aspect, the present invention provides the glycosylated dimers formed by disulfide bond at hinge region of two monomeric proteins whose concatamerized part involving immune response is fused to hinge region of Fc fragment of immunoglobulins.

Also in another aspect, the present invention provides the pharmaceutical compositions comprising dimers formed by disulfide bond at hinge region of two monomeric proteins whose concatamerized part involving immune response is fused to hinge region of Fc fragment of immunoglobulins in a pharmaceutically effective amount and in a pharmaceutically acceptable carrier.

Also in another aspect, the present invention provides the pharmaceutical compositions comprising glycosylated dimers formed by disulfide bond at hinge region of two monomeric proteins whose concatamerized part involving immune response is fused to hinge region of Fc fragment of immunoglobulins in a pharmaceutically effective amount and in a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Fig. 1 is a schematic view showing a process of preparing a DNA construct encoding a conventional simple fusion monomeric protein through polymerase chain reaction (PCR);

Fig. 2 is a schematic view showing a process of preparing a DNA construct encoding a concatameric fusion monomeric protein according to the present invention through PCR;

Fig. 3a shows structures of $[TNFR/Fc]_2$, $[CD2/Fc]_2$ or $[CTLA4/Fc]_2$ fusion proteins, which are simple fusion dimeric proteins formed through homodimerization in cells of TNFR/Fc, CD2/Fc or CTLA4/Fc fusion proteins as examples of conventional simple fusion monomeric proteins;

Fig. 3b shows structures of $[TNFR-TNFR/Fc]_2$, $[CD2-CD2/Fc]_2$ or $[CTLA4-CTLA4/Fc]_2$ fusion proteins, which are concatameric fusion dimeric proteins formed through homodimerization in cells of TNFR-TNFR/Fc, CD2-CD2/Fc or CTLA4-CTLA4/Fc fusion proteins as embodiments of the concatameric fusion dimeric protein according to the present invention;

Fig. 4a shows a structure of $[TNFR1-TNFR1/Fc]_2$, as an embodiment of a concatameric fusion dimeric protein according to the present invention;

Fig. 4b shows a structure of $[TNFR2-TNFR2/Fc]_2$, as another embodiment of the concatameric fusion dimeric protein according to the present invention;

Fig. 4c shows a structure of $[CD2-CD2/Fc]_2$, as a further embodiment of the concatameric fusion dimeric protein according to the present invention;

Fig. 4d shows a structure of $[CTLA4-CTLA4/Fc]_2$, as a still further embodiment of the concatameric fusion dimeric protein according to the present invention;

Fig. 5 is a diagram showing a process of constructing a recombinant expression plasmid pTR11Ig-Top10' expressing a concatameric fusion monomeric protein TNFR1-TNFR1/Fc according to the present invention;

Fig. 6 is a diagram showing a process of constructing a recombinant expression plasmid pCD22Ig expressing a concatameric fusion monomeric protein CD2-CD2/Fc according to the present invention;

Fig. 7 is a map of a recombinant expression plasmid pTR11Ig-Top10' expressing a concatameric fusion monomeric protein TNFR1-TNFR1/Fc according to the present invention;

Fig. 8 is a map of a recombinant expression plasmid pTR22Ig-Top10' expressing a concatameric fusion monomeric protein TNFR1-TNFR1/Fc according to the present invention;

Fig. 9 is a map of a recombinant expression plasmid pCD22Ig expressing a concatameric fusion monomeric protein CD2-CD2/Fc according to the present invention;

Fig. 10 is a map of a recombinant expression plasmid pCT44Ig expressing a concatameric fusion monomeric protein CTLA4-CTLA4/Fc according to the present invention;

5 Fig. 11 is a map of a recombinant expression plasmid pTR11Ig-MG expressing a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing four glycosylation motif peptides according to the present invention;

Fig. 12 is a map of a recombinant expression plasmid pTR22Ig-MG expressing a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing two glycosylation motif peptides according to the present invention;

10 Fig. 13 is a map of a recombinant expression plasmid pCD22Ig-MG expressing a concatameric fusion monomeric protein mgCD2-CD2/Fc containing two glycosylation motif peptides according to the present invention;

Fig. 14 is a map of a recombinant expression plasmid pCT44Ig-MG expressing a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing three glycosylation motif peptides according to the present invention;

15 Fig. 15 shows a result of SDS-PAGE of purified concatameric fusion dimeric proteins $[TNFR1-TNFR1/Fc]_2$ and $[TNFR2-TNFR2/Fc]_2$ under reducing or non-reducing conditions;

Fig. 16 is a graph showing inhibitory effect of the conventional simple fusion dimeric proteins $[TNFR1/Fc]_2$ (●) and $[TNFR2/Fc]_2$ (○) and the concatameric fusion dimeric proteins $[TNFR1-RNFR1/Fc]_2$ (▼) and $[TNFR2-TR2Fc]_2$ (▽) according to the present invention against cytotoxic activity of TNF-alpha;

20 Fig. 17 is a graph showing inhibitory effect of the conventional simple fusion dimeric proteins $[TNFR1/Fc]_2$ (●) and $[TNFR2/Fc]_2$ (○) and the concatameric fusion dimeric proteins $[TNFR1-RNFR1/Fc]_2$ (▼) and $[TNFR2-TR2Fc]_2$ (▽) according to the present invention against cytotoxic activity of TNF-beta;

25 Fig. 18 is a graph showing inhibitory effect of the conventional simple fusion dimeric protein $[CD2/Fc]_2$ (●), the known immunosuppressive agent cyclosporin A (▼) and the concatameric fusion dimeric protein $[CD2-CD2/Fc]_2$ (○) according to the present invention on the proliferation of active T lymphocytes;

30 Fig. 19 is a graph showing inhibitory effect of the conventional simple fusion

dimeric protein [CTLA4/Fc]₂(●), the known immunosuppressive agent cyclosporin A (▼) and the concatameric fusion dimeric protein [CTLA4-CTLA4/Fc]₂ (○) according to the present invention on the proliferation of active T lymphocytes;

Fig. 20 is a graph showing blood half-life of the conventional simple fusion dimeric protein [TNFR1/Fc]₂(●), the concatameric dimeric protein [TNFR1-TNFR1/Fc]₂ (○) and a glycosylated concatameric fusion dimeric protein [mgTNFR1-TNFR1/Fc]₂ (▼) according to the present invention;

Fig. 21 is a graph showing blood half-life of the conventional simple fusion dimeric protein [CD2/Fc]₂(●), the concatameric fusion dimeric protein [CD2-CD2/Fc]₂ (○) and a glycosylated concatameric fusion dimeric protein [mgCD2-CD2/Fc]₂ (▼) according to the present invention;

Fig. 22 is a graph showing blood half-life of the conventional simple fusion dimeric protein [CTLA4/Fc]₂(●), the concatameric fusion dimeric protein [CTLA4-CTLA4/Fc]₂ (○) and a glycosylated concatameric fusion dimeric protein [mgCTLA4-CTLA4/Fc]₂ (▼) according to the present invention; and

Fig. 23 is a graph showing inhibitory effect of PBS (●) as a control, the conventional simple fusion dimeric proteins [TNFR1/Fc]₂(■) and [TNFR2/Fc]₂(▲), and concatameric fusion dimeric proteins [TNFR1-TNFR1/Fc]₂(×) and [TNFR2-TNFR2/Fc]₂(△) according to the present invention on the induction of collagen-induced arthritis (CIA) in DBA/1 mice.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention is generally directed to concatameric proteins, and more particularly, to immunoadhesion molecules. Immunoadhesion molecules are typically formed by fusion of the Fc fragment of immunoglobulin (Ig) to a ligand-binding region of a receptor or an adhesion molecule, and thus have a structure similar to that of an antibody. The typical immunoadhesion molecules known in the art have a structure of an antibody in which the variable region is substituted with a ligand-binding region of a receptor while retaining the Fc fragment. A wide variety of immunoadhesion molecules are suggested in the literature. However, immunoadhesion molecules according to the

present invention have different structure with the conventional immunoadhesion molecules, and there is also no prior art predicting or describing preparation of the immunoadhesion molecules according to the present invention.

Definition of Terms

5 For full understanding of the characteristic structure of the immunoadhesion molecules according to the present invention, exact definitions of the terms used in the present invention are given as follows. In general, all of the technical and scientific terms being not additionally defined in the present invention have meanings commonly used in the art. However, although having meanings commonly used in the art, the
10 following terms are defined to give a clearer understanding of their meanings and make the scope of the present invention clear, as follows.

The term "immunoglobulin", as used herein, refers to protein molecules being produced in B cells and serving as antigen receptors specifically recognizing a wide variety of antigens. The molecules have a Y-shaped structure consisting of two identical
15 light chains (L chains) and two identical heavy chains (H chains), in which the four chains are held together by a number of disulfide bonds, including the disulfide bridge between the H chains at the hinge region. The L and H chains comprise variable and constant regions. The L chain variable region associates with the H chain variable region, thus producing two identical antigen-binding regions. According to features of the constant
20 regions of H chains, immunoglobulins (Ig) are classified into five isotypes, A (IgA), D (IgD), E (IgE), G (IgG) and M (IgM). Each subtype possesses unique structural and biological properties. For example, IgG has slightly different Fc structure, compared with other isotypes. In addition, IgG and IgA have a number of subtypes. For example, the human IgG isotype has four subtypes, IgG1, IgG2, IgG3 and IgG4, which
25 have $\gamma 1$, $\gamma 2$, $\gamma 3$ and $\gamma 4$ H chains, respectively. Biological functions of immunoglobulin molecules, such as complement activation, Fc receptor-mediated phagocytosis and antigen-dependent cytotoxicity, are mediated by structural determinants (complementarity-determining regions) in the Fc region of H chains. Such an Fc region of H chains is used for construction of dimeric proteins according to the present

invention, and may be derived from all isotypes and subtypes of immunoglobulin as described above.

5 The term "Fc fragment of an immunoglobulin molecule", as used herein, refers to a fragment having no antigen-binding activity and being easily crystallized, which comprises a hinge region and CH2 and CH3 domains, and a portion responsible for binding of an antibody to effector materials and cells. Therefore, the Fc fragment mentioned in the present invention can be different from that described in some literatures, but includes the hinge region. Such description of the Fc fragment is given to supply convenience in describing the present invention, and will be fully understood by those of ordinary skill in the art with reference to the specification of the present invention and the accompanying drawings.

10 The term "biologically active protein", as used herein, refers to a protein, peptide or polypeptide having generally physiological or pharmaceutical activities, which retains a part of its native activities after forming a concatamer or immunoadhesion molecule. The term "biological activity", as used herein, is not limited in meaning to physiological or pharmaceutical activities. For example, some concatamers, such as those containing an enzyme can catalyze a reaction in an organic solvent. Similarly, some high-molecular weight fusion molecules containing concanavalin A or an immunoglobulin molecule are useful as diagnostic agents in laboratories.

20 Non-limiting examples of the protein, peptide or polypeptide include hemoglobin, serum proteins (e.g., blood factors including factor VII, VIII and factor IX), immunoglobulin, cytokines (e.g., interleukin), α -, β - and γ -interferon, colony-stimulating agent (e.g., G-CSF and GM-CSF), platelet-derived growth factor (PDGF), and phospholipase activating proteins (PLAPs). Other typical biological or therapeutic proteins include insulin, plant proteins (e.g., lectin and ricin), tumor necrosis factor (TNF) and its related alleles, growth factors (e.g., tissue growth factors and endothelial growth factors such as TGF α or TGF β), hormones (e.g., follicle-stimulating hormone, thyroid-stimulating hormone, antidiuretic hormone, pigment-concentrating or dispersing hormones and parathyroid hormone, luteinizing hormone-releasing hormone and its derivatives, calcitonin, calcitonin gene related peptide (CGRP), synthetic enkephalin, somatomedin, erythropoietin, hypothalamus releasing factors, prolactin, chronic gonadotrophin, tissue

plasminogen-activating agents, growth hormone-releasing peptide (GHRP), and thymic humoral factor (THF). The immunoglobulins include IgG, IgE, IgM, IgA, IgD and fragments thereof. Some proteins such as interleukin, interferon or colony-stimulating factor may be produced in a non-glycosylated form using DNA recombinant techniques.

5 The non-glycosylated proteins may be useful as biologically active materials in the present invention.

In addition, the biologically active materials useful in the present invention include any polypeptide, which has bioactivity in vivo. Examples of the biologically active materials include peptides or polypeptides, fragments of an antibody, single chain-binding proteins (see U.S. Pat. No. 4,946,778), binding molecules including fusion polypeptides of antibodies or their fragments, polyclonal antibodies, monoclonal antibodies, and catalytic antibodies. Other examples of the biologically active materials include allergen proteins, such as ragweed, antigen E, honeybee venom, or allergen of mites.

15 In addition, the biologically active material useful in the present invention includes enzymes. Examples of the enzymes include carbohydrate-specific enzymes, proteinases, oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. In detail, non-limiting examples of the enzymes include asparaginase, arginase, arginine deaminase, adenosine deaminase, peroxide dismutase, endotoxinase, catalase, 20 chymotrypsin, lipase, uricase, adenosine dephosphatase, tyrosinase, and bilirubin oxidase. Examples of the carbohydrate-specific enzymes include glucose oxidase, glucodase, galactosidase, glucocerebrosidase, and glucouronidase.

The term "proteins involving immune response", as used herein, refers to all proteins mediating cell-to-cell signal transduction during cellular or humoral immune response and thus activating or suppressing immune response. Immunity is a process of protecting "self" from "non-self" such as bacteria or viruses. Immune response is largely divided into cellular and humoral immune response, where T and B lymphocytes play the most important role. T cells, mainly mediating cellular immune response, directly attack and kill virus-infected cells or tumor cells, or help other immune cells by secreting cytokines functioning to induce or activate immune response or inflammation. 30 B cells produce antibodies against non-self foreign materials (antigens) that enter a body,

such as bacteria or viruses, and such immune response is called cellular immune response. Cell-to-cell signal transduction is an essential process in both cellular and humoral immune responses, in which a signal molecule, that is, a ligand, interacts with a cell surface receptor acting to transduce a specific signal into a cell.

Representative examples of the proteins involving the immune response according to the present invention include cytokines, cytokine receptors, adhesion molecules, tumor necrosis factor receptor (TNFR), enzymes, receptor tyrosine kinases, chemokine receptors, other cell surface proteins, and soluble ligands. Non-limiting examples of the cytokines include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-17, TNF, TGF, IFN, GM-CSF, G-CSF, EPO, TPO, and M-CSF. Examples of the cytokine receptors, but are not limited to, include growth hormone receptors (GHRs), IL-13R, IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, IL-15R, TNFR, TGFR, IFNR (e.g., IFN- γ R α -chain and IFN- γ R β -chain), interferon- α R, β R and γ R, GM-CSFR, G-CSFR, EPOR, cMpl, gp130, and Fas (Apo 1). Non-limiting examples of the enzymes include influenza C hemagglutinin esterase and urokinase. The chemokine receptors are exemplified by CCR1 and CXCR1-4. Examples of the receptor tyrosine kinases, but are not limited to, include TrkA, TrkB, TrkC, Htk, REK7, Rse/Tyro-3, hepatocyte growth factor R, platelet-derived growth factor R, and Flt-1. Examples of other cell surface proteins includes CD2, CD4, CD5, CD6, CD22, CD27, CD28, CD30, CD31, CD40, CD44, CD100, CD137, CD150, LAG-3, B7, B61, β -neurexin, CTLA-4, ICOS, ICAM-1, complement R-2 (CD21), IgER, lysosomal membrane gp-1, α 2-microglobulin receptor-related proteins, and sodium-releasing peptide R. Non-limiting examples of the soluble ligands include IL-10, heregulin, and keratinocyte growth factors.

Ligands for the proteins involving immune response according to the present invention and use thereof are well known to those of ordinary skill in the art, as summarized in Tables 1 to 7, below.

TABLE 1
Proteins involving immune response: Adhesion molecules

Adhesion molecules	Ligands	Uses
CD4	HIV gp120	Inhibition of in vivo HIV infection; and identification of CD4 domain participating in ligand binding
L-Selectin	GlyCAM-1, CD34	Prevention of neutrophil-mediated lung damage; determination of position in tissues of a ligand by histochemical staining; and isolation and cloning of ligands and determination of their properties
E-Selectin	Sialyl Lewis ^x	Prevention of neutrophil-mediated lung damage; and determination of thermodynamic properties in ligand-binding
P-Selectin	Sialyl Lewis ^x	Prevention of neutrophil-mediated lung damage; and study of functions of individual of amino acid residues in binding to cell surface
ICAM-1	CD11a/CD18	Phagocytosis of erythrocytes in malaria; inhibition of infection with rhinovirus; and anti-inflammation in diabetes
ICAM-2	CD11a/CD18	Study of activation of T cells mediated by T cell receptor
ICAM-3	CD11a/CD18	Identification of receptor domains binding to a ligand
VCAM-1	VLA-4	Study of role of VLA-4 in T lymphocyte migration to dermal inflammation sites
LFA-3	CD2	Study of role of CD2 in costimulation of T cells
L1 glycoprotein	Fibroblast growth factor receptor	Stimulation of nerve reproduction after repair; and functional comparison with FGF

TABLE 2
Proteins involving immune response: Enzymes

Enzymes	Ligands	Uses
Influenza C hemagglutinin esterase	9-O-acetylated sialic acid	Inactive enzyme used in study of tissue-specific expression of ligands
Urokinase	Urokinase receptor	Inactive enzyme developed to inhibit cancer metastasis by disturbing urokinase activation

TABLE 3
Proteins involving immune response: Cytokine receptors

Cytokine receptors	Ligands	Uses
IFN- γ R α -chain	IFN- γ	Inhibition of IFN-mediated autoimmunity
IFN- γ R β -chain	IFN- γ	Study of structure of subunits of a ligand-receptor complex
IL1R	IL-1	Inhibition of IL-1-mediated inflammation
IL4R	IL-4	Identification of receptor domains participating in ligand binding
Erythropoietin R	Erythropoietin	Map design of epitopes of anti-ligand antibodies
cMpl	Thrombopoietin	Isolation and cloning of ligands
gp130	IL-6-IL6R complex	Study of structure of subunits of a ligand-receptor complex

TABLE 4

Proteins involving immune response: Tumor necrosis factor receptors

TNF receptors	Ligands	Uses
TNF R-1	TNF, lymphotoxin- α	Treatment of septic shock, rheumatoid arthritis and other inflammatory diseases; and identification of domains participating in ligand binding
TNF R-2	TNF, lymphotoxin- α	Inhibition of TNF-enriched HIV replication; and prevention of collagen-induced arthritis in mice
Lymphotoxin- β R	Lymphotoxin- β	Study of structure of subunits of cell surface lymphotoxin- β
Fas/Apo-1/CD95	Fas/Apo-1/CD95 ligand	Treatment of excessive apoptosis and related diseases (e.g., AIDS); and resistance to apoptosis of lymphocytes and peripheral immune tolerance; roles of Fas ligand in T cell-mediated cytotoxicity; and isolation and cloning of ligands
CD27	CD27 ligand	Isolation and cloning of ligands
CD30	CD30 ligand	Isolation and cloning of ligands
CD40	gp39	Isolation and cloning of ligands
4-1BB	4-1BB ligand	Identification of tissues containing ligands by histochemical staining; isolation and cloning of ligands; and Study of structural determinant of potential ligand
OX40	gp34	Isolation and cloning of ligands

TABLE 5

Proteins involving immune response: Receptor tyrosine kinases

Receptor tyrosine kinases	Ligands	Uses
TrkA, B, C	Neutropin	Determination of properties of neutropin binding
Htk	Htk ligand	Isolation and cloning of ligands
REK7	AL-1	Isolation and cloning of ligands
Rse/Tyro-3	Protein S, Gas6	Identification of ligands and determination of their properties
Hepatocyte growth factor R	Hepatocyte growth factor	Identification of receptor domains participating in ligand binding
Platelet-derived growth factor R	Platelet-derived growth factor	Identification of receptor domains participating in ligand binding
Fli-1	Vesicular endothelial growth factor (VEGF)	Determination of properties of ligand binding of receptors
Fli-1/KDR	VEGF	Evaluation of selectivity of receptors for VEGF versus placenta growth factor

TABLE 6
Proteins involving immune response: Other cell surface proteins

Other cell surface proteins	Ligands	Uses
B7	CD28	Study of T cell stimulation by B cells
B61	Eck	Roles of Eck in inflammation
β -neurexin	β -neurexin ligand	Determination of properties of a signal sequence from β -neurexin
CD2	LFA-3, CD48	Identification of ligands
CD5	CD5 ligand	Study of T cell stimulation by B cells
CD6	ALCAM	Study of binding activities of cloned ligands
CD22	CD45, other sialoglycoproteins	Identification of ligands; study on roles of CD22 in T-B-cell interaction; and determination of properties of binding determinants of sialo-oligo sugar ligands
CD28	B7, B7-2	Study of T cell stimulation by B cells
CD31	CD31	Identification of CD31 domains related to homotypic binding
CD44	Hyaluronate	Screening of tissues containing ligands by histochemical staining; and determination of properties of structural determinants of ligands
Complement R-2 (CD21)	C3 fragment	Inhibition of reactivity of antibody to immunosuppressive and cancer therapeutic agents
CTLA-4	B7	Identification of CTLA-4 as a secondary receptor of B7
IgE R	IgE	Inhibition of mast cell-binding of IgE as therapy of allergic diseases
Lysosome membrane gp-1	LAMP-1 ligand	Design of epitope maps of anti-ligand antibodies
α 2-microglobulin receptor-bound proteins	gp330	Determination of position of ligands in tissues by histochemical staining
Sodium-releasing peptide R	Sodium-releasing peptide	Design of epitope maps of anti-ligand antibodies; and preparation of recombinant receptors for structural study

TABLE 7
Proteins involving immune response: Soluble ligands

Soluble ligands	Ligands	Uses
IL-2	IL-2R	Extension of half-life of IL-2 in the circulation system
IL-10	IL-10R	Therapy of septic shock and transplantation rejection; and extension of half-life of IL-10 in the circulation system
Heregulin	Her4/p180 ^{erbB4}	Study of signal transduction by Her4
Keratinocyte growth factor	Keratinocyte growth factor R	Determination of position of receptors by histochemical staining

5

The term "soluble extracellular domain", as used herein, refers to a portion exposed to the extracellular region of an integral membrane protein penetrating the cell membrane comprising phospholipid, wherein the integral membrane protein contains one or more transmembrane domain made up predominantly of hydrophobic amino acids.

Such an extracellular domain mainly comprises hydrophilic amino acids, which are typically positioned at the surface of a folded structure of a protein, and thus is soluble in an aqueous environment. For most cell surface receptor proteins, extracellular domains serve to bind specific ligands, while intracellular domains play an important role in signal transduction.

The term "concatamer-linked", as used herein, refers to a state in which two soluble domains of biologically active proteins are linked and thus form a long polypeptide.

The term "concatameric protein", as used herein, means a concatamer-linked protein. For example, the N-terminus of a soluble extracellular domain of a protein involving immune response is linked to the C-terminus of an identical soluble extracellular domain of the protein involving immune response, wherein the C-terminus of the former soluble extracellular domain is linked to the hinge region of an Fc fragment of an immunoglobulin molecule. Thus, two identical soluble extracellular domains of a protein involving immune response form a long polypeptide.

The term "simple fusion monomeric protein", as used herein, refers to a fusion protein having a monomeric structure consisting of a single polypeptide formed by linkage of a soluble extracellular domain of a protein involving immune response to the hinge region of an Fc fragment of an immunoglobulin molecule. A simple fusion monomeric protein may be designated "protein name/Fc" for convenience in the present invention. For example, a simple fusion monomeric protein produced by linkage of an soluble extracellular domain of TNFR1 protein involving immune response to an Fc fragment of an immunoglobulin molecule is designated TNFR1/Fc. If desired, the origin of the Fc fragment may be also specified in the designation. For example, in the case that the Fc fragment is derived from IgG1, the monomeric protein is called TNFR1/IgG1Fc.

The term "simple fusion dimeric protein", as used herein, refers to a fusion protein having a dimeric structure, in which two simple fusion monomeric proteins are joined by formation of intermolecular disulfide bonds at the hinge region. Such a simple fusion dimeric protein may be designated "[protein name/Fc]₂" for convenience in the present invention. For example, when fused by formation of intermolecular disulfide

bonds at the hinge region of two simple fusion monomeric proteins produced by linkage of an soluble extracellular domain of TNFR1 protein and an Fc fragment of an immunoglobulin molecule, the resulting fusion protein having dimeric structure is designated [TNFR1/Fc]₂. In addition, the origin of the Fc fragment may be specified in the designation, if desired. For example, in the case that the Fc fragment is derived from IgG1, the dimeric protein is designated [TNFR1/IgG1Fc]₂.

The term "concatameric fusion monomeric protein", as used herein, refers to a fusion protein having a monomeric structure consisting of a single polypeptide, in which the N-terminus of a soluble extracellular domain of a protein involving immune response is linked to the C-terminus of an identical soluble extracellular domain of the protein involving immune response, wherein the C-terminus of the former soluble extracellular domain is linked to the hinge region of an Fc fragment of an immunoglobulin molecule. A concatameric fusion monomeric protein may be designated "protein name-protein name/Fc" for convenience in the present invention. For example, when an soluble extracellular domain of TNFR1 of a simple fusion monomeric protein, produced by linkage of the soluble extracellular domain of TNFR1 protein involving immune response and an Fc fragment of an immunoglobulin molecule, is linked to an identical soluble extracellular domain of TNFR1, the resulting concatameric fusion monomeric protein is designated TNFR1-TNFR1/Fc. If desired, the origin of the Fc fragment may be specified in the designation. For example, in the case that the Fc fragment is derived from IgG1, the monomeric protein is designated TNFR1-TNFR1/IgG1Fc.

The term "concatameric fusion dimeric protein", as used herein, refers to a fusion protein having a dimeric structure, in which two concatameric fusion monomeric proteins are fused by formation of intermolecular disulfide bonds at the hinge region. A concatameric fusion dimeric protein may be designated "[protein name-protein name/Fc]₂" for convenience in the present invention. For example, when two concatameric fusion monomeric proteins, each of which is produced by linkage of a TNFR1 soluble extracellular domain of a simple fusion monomeric protein to an identical soluble extracellular domain of TNFR1 protein involving immune response, are fused by formation of intermolecular disulfide bonds at the hinge region, the resulting fusion protein having dimeric structure is designated [TNFR1-TNFR1/Fc]₂, wherein the simple

fusion monomeric protein is formed by linkage of the TNFR1 soluble extracellular domain to an Fc fragment from an immunoglobulin molecule. If desired, the origin of the Fc fragment may be specified in the designation. For example, in the case that the Fc fragment is derived from IgG1, the fusion protein is designated [TNFR1-IgG1Fc]₂.

The term "vector", as used herein, means a DNA molecule serving as a vehicle capable of stably carrying exogenous genes into host cells. For useful application, a vector should be able to replicate, have a system for introducing itself into a host cell, and possess selectable markers. The exogenous genes, for example, include, a DNA construct encoding a concatameric fusion monomeric protein.

The term "recombinant expression plasmid", as used herein, refers to a circular DNA molecule carrying exogenous genes operably linked thereto to be expressed in a host cell. When introduced into a host cell, the recombinant expression plasmid has the ability to replicate regardless of host chromosomal DNA, copy itself at a high copy number, and to produce heterogeneous DNA. As generally known in the art, in order to increase the expression level of a transfected gene in a host cell, the gene should be operably linked to transcription and translation regulatory sequences functional in a host cell selected as an expression system. Preferably, the expression regulation sequences and the exogenous genes may be carried in a single expression vector containing bacteria-selectable markers and a replication origin. In case that eukaryotic cells are used as an expression system, the expression vector should further comprise expression markers useful in the eukaryotic host cells.

The term "operably linked", as used herein, means an arrangement of elements of a vector, in which each element is capable of performing its innate function. Therefore, a control sequence operably linked to a coding sequence can influence expression of the coding sequence. A control sequence acting to induce expression of a coding sequence does not have to be adjacent to the coding sequence. For example, when an intervening sequence is present between a promoter sequence and a coding sequence, the promoter sequence may still be "operably linked" to the coding sequence.

Host cells used in the present invention may be prokaryotic or eukaryotic. In addition, host cells having high introduction efficiency of foreign DNA and having high

expression levels of an introduced gene may be typically used. Examples of the host cells useful in the present invention include prokaryotic and eukaryotic cells such as *E. coli*, *Pseudomonas* sp., *Bacillus* sp., *Streptomyces* sp., fungi or yeast, insect cells such as *Spodoptera frugiperda* (Sf9), animal cells such as Chinese hamster ovary cells (CHO) or mouse cells, African green monkey cells such as COS 1, COS 7, human embryonic kidney cells, BSC 1, BSC 40 or BMT 10, and tissue-cultured human cells. When cloning a DNA construct encoding the fusion protein according to the present invention, host cells are preferably animal cells. When using COS cells, since SV40 large T antigen is expressed in COS cells, a plasmid carrying a SV 40 replication origin may be present as a multicopy episome and thus allows high expression of an exogenous gene. A DNA sequence introduced into a host cell may be homogeneous or heterogeneous to the host cell, or a hybrid DNA sequence containing a homogenous or heterogeneous DNA sequence.

In order to express a DNA sequence encoding the concatameric fusion protein according to the present invention, a wide variety of combinations of host cells as an expression system and vectors may be used. Expression vectors useful for transforming eukaryotic host cells contain expression regulation sequences from, for example, SV40, bovine papillomavirus, adenovirus, adeno-associated viruses, cytomegalovirus and retroviruses. Expression vectors useful in bacterial host cells include bacterial plasmids from *E. coli*, which are exemplified by pBluescript, pGEX2T, pUC, pCR1, pBR322, pMB9 and derivatives thereof, plasmids having a broad range of host cells, such as RP4, phage DNAs, exemplified by a wide variety of λ phage derivatives including λ gt10, λ gt11 and NM989, and other DNA phages, exemplified by filamentous single-stranded DNA phages such as M13. Expression vectors useful in yeast cells include 2μ plasmid and derivatives thereof. Expression vectors useful in insect cells include pVL 941.

The term "transformation", as used herein, means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration.

The term "transfection", as used herein, refers to the taking up of an expression vector by a suitable host cell, whether any coding sequences are in fact expressed or not.

The term "signal sequence", as used herein, means an amino acid sequence mediating transport of an expressed protein to the outside of the cell membrane, and is also

called a "leader sequence". Cell surface proteins or secretory proteins, which are transported to the outside of the cell membrane, have an N-terminal sequence typically cut by signal peptidase in the cell membrane. Such a N-terminal sequence is called a signal sequence or signal peptide, or a leader sequence or leader peptide. Secretory (or transported) proteins or all proteins present outside of the cell membrane or in the extracellular environment have a specific signal sequence. There is no specific homology between such signal sequences and same proteins have different signal sequences according to their origin. Secondary structure or distribution of nonpolar and charged residues is more important for proper function of the signal sequences than primary structures thereof.

Although not having specific homology, the signal sequences share several common features, as follows. The signal sequences contain an N domain at their N-termini, which is a hydrophilic region comprising one or more positively charged residues, and an H-domain follows the N domain, which is a somewhat long hydrophobic region. In the case of *E. coli*, the signal sequence comprises about 18-30 amino acids. The N domain contains many cationic amino acids such as Lys or Arg, and thus has a net positive charge. Many hydrophobic amino acids such as Ala or Leu are found in the H domain, and polar or charged amino acids such as Pro, Lys, Arg, Asn or Glu are rarely in the H domain. A large number of amino acids such as Ala and Leu residues form an α -helical structure to facilitate membrane penetration. A C domain is positioned between the H domain and an actually secreted portion of a protein. The C domain is less hydrophobic, and contains a sequence capable of being recognized by signal peptidase such as LebB or LspA. There have been no reports about an exact site cleaved by the signal peptidase, but the signal peptidase is typically known to mostly cleave behind the Ala-X-Ala sequence in the C domain. Preproteins containing the above-mentioned signal sequence arrive at the cell membrane through interaction with several proteins, and fold to their mature forms through cleavage of a specific region of a signal peptide. Such a signal sequence is very important in strategies to express a desired protein on the cell surface or in the extracellular environment. Foreign proteins and fusion proteins should be stably transported to the extracellular environment at high efficiency. Typically, cell surface proteins having excellent secretory ability are useful for cell surface expression of foreign proteins or fusion

proteins, which typically have secretory signal sequences capable of offering excellent secretion efficiency.

Preparation of the concatameric fusion dimeric protein according to the present invention

5 The concatameric fusion dimeric protein according to the present invention is generally prepared by (a) preparing a DNA construct encoding a simple fusion monomeric protein using a gene encoding an Fc fragment of an immunoglobulin molecule and a gene encoding a soluble extracellular domain of a protein involving immune response; (b) inserting by polymerase chain reaction (PCR) a recognition sequence of a restriction
10 enzyme into the prepared simple fusion monomeric protein-encoding DNA construct and an identical gene to the gene encoding a soluble extracellular domain of a protein involving immune response, respectively; (c) cleaving the recognition sequence of a restriction enzyme in the simple fusion monomeric protein-coding DNA construct and the gene encoding a soluble extracellular domain of a protein involving immune response using the
15 restriction enzyme recognizing the recognition sequence; (d) ligating the cleaved DNA fragments using ligase to produce a DNA construct encoding a concatameric fusion monomeric protein (see, Fig. 2); (e) operably linking the prepared DNA construct encoding a concatameric fusion monomeric protein to a vector to produce a recombinant expression plasmid; (f) transforming or transfecting a host cell with the recombinant expression
20 plasmid; and (g) culturing the transformant or transfectant under conditions suitable for expression of the DNA construct encoding a concatameric fusion monomeric protein and then isolating and purifying a concatameric fusion dimeric protein of interest.

25 A DNA fragment encoding a soluble extracellular domain of a protein involving immune response is produced by PCR using a primer containing a recognition sequence of a specific restriction enzyme and a sequence encoding a leader sequence, and a primer containing an antisense sequence encoding the 3' end of the soluble extracellular domain and a portion of the 5' end of a specific region of Fc fragment of an immunoglobulin molecule.

A DNA fragment encoding a specific region of the Fc fragment of an immunoglobulin molecule is produced by PCR using a primer having a sequence encoding a portion of the 3' end of the soluble extracellular domain of the protein involving immune response and a sequence encoding the 5' end of the specific region of the Fc fragment of an immunoglobulin molecule, and another primer having an antisense sequence encoding a recognition sequence of a specific restriction enzyme and the 3' end of a specific region of the Fc fragment of an immunoglobulin molecule.

The DNA fragment encoding a soluble extracellular domain of a protein involving the immune response and the DNA fragment encoding a specific region of Fc fragment of an immunoglobulin molecule, as described above, are mixed in a test tube. After denaturation, the DNA is re-annealed. Then, a complete double-stranded DNA fragment is produced by polymerization using DNA polymerase at the 3' end of each DNA hybrid. Using the resulting double-stranded DNA fragment, another polymerase chain reaction (PCR) is carried out with the primer having a sequence encoding a soluble extracellular domain of a protein involving immune response and the primer encoding the 3' end of a specific region of the Fc fragment of an immunoglobulin molecule, in order to amplify a immunoglobulin fusion gene comprising a sequence corresponding to the DNA fragment encoding a soluble extracellular domain of a protein involving immune response and a sequence corresponding to the DNA fragment encoding a specific region of the Fc fragment of an immunoglobulin molecule.

An recognition sequence of a restriction enzyme is introduced by PCR into the amplified immunoglobulin fusion gene and the DNA fragment having a sequence encoding a soluble extracellular domain of a protein involving the immune response. The recognition sequence is then cleaved with the restriction enzyme and the cleaved regions are ligated using ligase, thus producing a concatameric immunoglobulin fusion gene.

The immunoglobulin fusion gene may further include a signal sequence to stimulate extracellular secretion of a protein encoded thereby. For example, the CTLA-4 molecule contains a unique leader sequence having highly hydrophilic redundancy at its N-terminus, and which is abnormally long and highly water-soluble (Harper, K. et al., J. Immunol. 147:1037-1044; and Brunet, J.F. Nature 328:267-270, 1987). Generally, most

cell surface proteins or secretory proteins have a leader sequence comprising 20-24 highly hydrophobic amino acids at their N-termini. However, the CTLA-4 molecule used in the present invention comprises a total of 37 residues: 16 hydrophilic amino acids at its N-terminus, and 21 highly hydrophobic amino acids typical in its transmembrane regions.

5 In the conventional method of preparing CTLA4Ig fusion proteins, the leader sequence of the CTLA-4 molecule was substituted with a leader sequence of oncostatin M (Linsley, P.S. et al., J. Exp. Med. 174:561-569, 1991) or IL-6 (Yamada, A, et al., Microbiol. Immunol. 40:513-518, 1996). The present inventors demonstrated that a CTLA-4 molecule containing a leader sequence having a "MRTWPCTLLFFIPVFCKA" sequence
10 instead of the amino acid sequence consisting of 16 amino acids, "ACLGFRHKAQKNLAA", is preferable, and the secretion of an expressed protein to the extracellular environment is easily achieved, as disclosed in International Pat. Publication No. WO98/31820.

A recombinant expression plasmid is prepared by inserting the immunoglobulin
15 fusion gene into a vector, and then introduced to a host cell to produce a transformant or transfectant. A concatameric fusion dimeric protein of interest may be obtained by culturing the transformant or transfectant cell and isolating and purifying a concatameric fusion protein.

A host cell useful for preparation of the concatameric fusion dimeric protein
20 according to the present invention is preferably selected from among bone marrow cell lines, CHO cells, monkey COS cells, human embryonic kidney 293 cells, and baculovirus-infected insect cells. A polypeptide of interest, produced in such an expression system, is secreted to culture medium as an inclusion body. Then, the concatameric fusion dimeric protein can be purified by affinity chromatography using a
25 protein A or protein G column. In fact, effective mammalian expression systems and such purification systems are very useful in expressing proteins involving immune response in a dimeric form, and isolation of such proteins.

Preparation of the glycosylated concatameric fusion dimeric protein according to the present invention

Secretory proteins produced in eukaryotic cells as host cells are modified by glycosylation. Glycosylation is known to influence in vivo stability and functionality as well as physical properties of a protein. Therefore, a preferred aspect of the present invention includes facilitating production of a concatameric fusion dimeric protein of interest using recombinant DNA techniques and the above-mentioned animal cell lines as host cells, and linking additional sugar chains to a soluble extracellular domain of a protein involving immune response.

Two glycosylation patterns are known. One is O-linked glycosylation, in which an oligosaccharide is linked to a serine or threonine residue, and the other is N-linked glycosylation, in which an oligosaccharide is linked to asparagine residue. N-linked glycosylation occurs at a specific amino acid sequence, particularly, Asn-X-Ser/Thr, wherein X is any amino acid excluding proline. N-linked oligosaccharide has a structure distinct from O-linked oligosaccharide, and glycosylated residues found in the N-linked type also differ from the O-linked type. For example, N-acetylgalactosamine is invariably linked to serine or threonine in O-linked oligosaccharide, while N-acetylglucosamine is linked to asparagines in all of N-linked oligosaccharides. The O-linked oligosaccharides generally contain only 1-4 sugar residues. In contrast, the N-linked oligosaccharides comprise 5 or more sugar residues, essentially including N-acetylglucosamine and mannose.

In accordance with the present invention, to allow additional O-linked or N-linked glycosylation, one or more nucleotides in a DNA sequence encoding a soluble extracellular domain of a protein involving immune response are altered, and the resulting DNA is expressed in a suitable animal host cell to induce glycosylation using the host system. In accordance with an aspect of the present invention, the glycosylated concatameric fusion dimeric protein according to the present invention may be prepared by altering a DNA sequence encoding a soluble extracellular domain of a protein involving immune response to induce or increase N-linked glycosylation by adding the sequence Asn-X-Ser/Thr.

Alteration of a DNA sequence to introduce glycosylation may be performed according to the conventional method common in the art. In a preferred aspect of the present invention, to protect the concatameric fusion protein, especially the two soluble

extracellular domains, from attack of intercellular proteinases and thus increase its half-life in serum, a DNA construct encoding a multiglycosylated concatameric fusion monomeric protein may be prepared using PCR, which introduces multiglycosylation sites to the joint region between two soluble extracellular domains. In a specific aspect of the present invention, glycosylation motif peptide sequences may be introduced into the concatameric fusion protein, as follows. A DNA fragment is prepared by performing PCR using a primer encoding a leader sequence of a soluble extracellular domain and EcoRI restriction site, and an antisense primer in which a portion of a nucleotide sequence encoding a portion of the 3' end of a first soluble extracellular domain and a portion of the 5' end of a second soluble extracellular domain is substituted with glycosylation motif sequences. Another DNA fragment is prepared by performing PCR using a primer in which a portion of a nucleotide sequence encoding a portion of the 3' end of a first soluble extracellular domain and a portion of the 5' end of a second soluble extracellular domain is substituted with glycosylation motif sequences, and an antisense primer encoding the 3' end of Fc portion of IgG1 and XbaI restriction site. Then, secondary PCR is carried out in a test tube using the two DNA fragments.

In accordance with an embodiment of the present invention, the soluble extracellular domains useful in the present invention include soluble extracellular domains of TNFR1, TNFR2, CD2 and CTLA-4. Their application will be described in detail with reference to accompanying figures, sequence listing and examples.

Tumor necrosis factor-alpha (TNF- α), which is known as the hormone cachectin, and tumor necrosis factor-beta (TNF- β), which is also known as lymphotoxin, are multifunctional cytokines, inducing inflammation, cellular immune response, septicemia, cytotoxicity, cachexia, rheumatoid arthritis, inflammation-related diseases (Tartaglia, L.A. et al., Immunol. Today 13:151,1992), and antiviral reaction (Butler, P., Peptide Growth Factor II, 1990, Springer-Verlag, Berlin, pp.39-70). Such actions of TNF- α and TNF- β , including cytotoxic activity, originate from their binding to TNF receptors in a trimeric form (Eck, M.J. et al., J. Biol. Chem. 267:2119, 1992). As TNF receptors, 55 kDa-type I (TNFR1 or p55) and about 75 kDa-type II (TNFR2 or p75) are known (Smith, C.A. et al., Science 248:1019, 1990; Loetscher, H. et al., Cell 61:351, 1990; and Schall et al., Cell 61:361, 1990). The two receptors have similar affinity for TNF- α and TNF- β (Schall et

al., Cell 61:361, 1990). Immunoglobulin fusion proteins of such soluble receptors have effects of inhibiting the action of TNF- α and TNF- β by inhibiting binding of TNF- α and TNF- β to their receptors on the cell surface, which is known to be effective in reducing TNF-dependent inflammation.

5 Among cell surface antigens regulating immune response, the costimulatory molecule CD2 and CTLA-4, inducing secondary stimulation to give sufficient activation of T cells, when being in a soluble form, also can be used for therapy of diverse immunological diseases according to the same method as TNF receptors. Immune response is accomplished by binding of cell surface antigen molecules of antigen
10 presenting cells (APC) to specific receptors of T lymphocytes, that is, T lymphocytes and leukocyte-function-antigen molecules of APC, and when a costimulatory signal as a secondary signal is not produced during antigen-presenting, T lymphocytes are removed by apoptosis or inhibition of clonal activation. CD2 is a leukocyte-function-antigen on T lymphocytes, binding to LFA-3 on APC, and participates in adhesion and costimulation
15 of leukocytes, as well as stimulating T cell activation through costimulation with CD28. CTLA-4 is expressed after activation of T lymphocytes, and its expression level is increased in the resting phase. CTLA-4 has a binding affinity to the B7 molecule of APC over 20 times higher than that of CD28, and transduces signals inhibiting T lymphocyte activation after binding to B7.

20 In a specific aspect of the present invention, there are provided a concatameric fusion monomeric protein TNFR1-TNFR1/Fc, designated by SEQ ID NO: 6; a concatameric fusion monomeric protein TNFR2-TNFR2/Fc, designated by SEQ ID NO: 8; a concatameric fusion monomeric protein CD2-CD2/Fc, designated by SEQ ID NO: 18; and a concatameric fusion monomeric protein CTLA4-CTLA4/Fc, designated by SEQ
25 ID NO: 20.

In another specific aspect of the present invention, there are provided a DNA construct (TNFR1-TNFR1-IgG) encoding a concatameric fusion monomeric protein TNFR1-TNFR1/Fc, designated by SEQ ID NO: 5; a DNA construct (TNFR2-TNFR2-IgG) encoding a concatameric fusion monomeric protein TNFR2-TNFR2/Fc, designated
30 by SEQ ID NO: 7; a DNA construct (CD2-CD2-IgG) encoding a concatameric fusion monomeric protein CD2-CD2/Fc, designated by SEQ ID NO: 17; and a DNA construct

(CTLA4-CTLA4-IgG) encoding a concatameric fusion monomeric protein CTLA4-CTLA4/Fc, designated by SEQ ID NO: 19.

In a further specific aspect of the present invention, there are provided a recombinant expression plasmid pTR11Ig-Top10' operably linked to a DNA construct
5 encoding a concatameric fusion monomeric protein TNFR1-TNFR1/Fc, designated by SEQ ID NO: 5; a recombinant expression plasmid pTR22Ig-Top10' operably linked to a DNA construct encoding a concatameric fusion monomeric protein TNFR2-TNFR2/Fc, designated by SEQ ID NO: 7; a recombinant expression plasmid pCD22Ig operably linked to a DNA construct encoding a concatameric fusion monomeric protein CD2-
10 CD2/Fc, designated by SEQ ID NO: 17; and a recombinant expression plasmid pCT44Ig operably linked to a DNA construct encoding a concatameric fusion monomeric protein CTLA4-CTLA4/Fc, designated by SEQ ID NO: 19. The recombination expression plasmids are deposited in Korean Culture Center of Microorganisms (KCCM) and are assigned accession Nos. KCCM-10288, KCCM-10291, KCCM-10402 and KCCM-10400,
15 respectively. The KCCM deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

In a further specific aspect of the present invention, there are provided a mammalian host cell (e.g., TR11Ig-CHO) transformed or transfected with a recombinant
20 expression plasmid pTR11Ig-Top10' operably linked to a DNA construct encoding a concatameric fusion monomeric protein TNFR1-TNFR1/Fc, designated by SEQ ID NO: 5; a mammalian host cell (e.g., TR22Ig-CHO) transformed or transfected with a recombinant expression plasmid pTR22Ig-Top10' operably linked to a DNA construct encoding a concatameric fusion monomeric protein TNFR2-TNFR2/Fc, designated by
25 SEQ ID NO: 7; a mammalian host cell transformed or transfected with a recombinant expression plasmid pCD22Ig operably linked to a DNA construct encoding a concatameric fusion monomeric protein CD2-CD2/Fc, designated by SEQ ID NO: 17; and a mammalian host cell transformed or transfected with a recombinant expression plasmid pCT44Ig operably linked to a DNA construct encoding a concatameric fusion
30 monomeric protein CTLA4-CTLA4/Fc, designated by SEQ ID NO: 19. Chinese hamster ovary cell line TR11Ig-CHO transfected with the recombinant expression

plasmid pTR11Ig-Top10' and Chinese hamster ovary cell line TR22Ig-CHO transfected with the recombinant expression plasmid pTR22Ig-Top10' are deposited in KCCM and are assigned accession Nos. KCLRF-BP-00046 and KCLRF-BP-00049, respectively. The KCCM deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

In a still further specific aspect of the present invention, there are provided a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 10; a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 12; a concatameric fusion monomeric protein mgCD2-CD2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 22; and a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 24.

In a still further specific aspect of the present invention, there are provided a DNA construct encoding a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 9; a DNA construct encoding a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 11; a DNA construct encoding a concatameric fusion monomeric protein mgCD2-CD2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 21; and a DNA construct encoding a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 23. In order to produce a glycosylation motif peptide, a primer set (forward and reverse primers) is designed, which are complementary to a nucleotide sequence corresponding to the joint region between soluble extracellular domains of concatameric fusion proteins of TNFR/Fc, CD2/Fc and CTLA4/Fc, as well as containing codons encoding asparagine (N) (ATT and AAC) or codons encoding serine (S) and threonine (T) (TCC, and ACC, ACG and ACA, respectively), with which any codon in the concatameric fusion protein gene may be substituted. When designing the primer, selection of one among a plurality of amino acid sequences may be determined

depending on a condition allowing minimum substitution of the nucleotide sequence and melting temperature (T_m) of each primer.

In a still further specific aspect of the present invention, there are provided a recombinant expression plasmid pTR11Ig-MG operably linked to a DNA construct
5 encoding a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 9; a recombinant expression plasmid pTR22Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 11; a recombinant expression plasmid pCD22Ig-MG operably
10 linked to a DNA construct encoding a concatameric fusion monomeric protein mgCD2-CD2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 21,; and a recombinant expression plasmid Pct44Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 23. The recombination
15 expression plasmids are deposited in Korean Culture Center of Microorganisms (KCCM) and are assigned accession Nos. KCCM-10404, KCCM-10407, KCCM-10401 and KCCM-10399, respectively. The KCCM deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

In a still further specific aspect of the present invention, there are provided a
20 mammalian host cell transformed or transfected with a recombinant expression plasmid pTR11Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 9; a mammalian host cell transformed or transfected with a
25 recombinant expression plasmid pTR22Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 11; a mammalian host cell transformed or transfected with a recombinant expression plasmid pCD22Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgCD2-
30 CD2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 21; and a mammalian host cell transformed or transfected with a recombinant expression plasmid

Pct44Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 23.

5 The concatameric fusion dimeric proteins of the present invention may be isolated from culture medium after culturing the transformants or transfectants according to the present invention. The concatameric fusion dimeric proteins may participate in immune response, as described in Table 1, above, and are thus useful as therapeutic agents, diagnostic agents and laboratory tools according to the kinds of the protein, and their use is well known to those of ordinary skill in the art. In particular, when being
10 used as therapeutic agents, the concatameric fusion dimeric proteins may be applied at an therapeutically effective amount common in the art, and it will be understood that such an amount may vary depending on diverse factors including activity of the used compound, patient's age, body weight, health state, sex and diet, administration time, administration route, combination of drugs, and pathogenic state of a specific disease to be prevented or
15 treated. In addition, when being used as therapeutic agents, it will be understood that the concatameric fusion dimeric proteins according to the present invention may be applied by the typical methods and routes for administration of proteins involving immune response, which are known to those of ordinary skill in the art.

20 The present invention will be explained in more detail with reference to the following examples in conjunction with the accompanying drawings. However, the following examples are provided only to illustrate the present invention, and the present invention is not limited to them. For convenience in describing the present invention, information on DNA constructs, recombinant expression plasmids and transformed cell lines, which are prepared according to the Examples, below, and the used primers and
25 accession numbers is summarized in Tables 8 and 9, below.

TABLE 8
Information on DNA constructs and accession Nos.

DNA construct name	SEQ ID No.		Deposition of genes		Deposition of cell lines	
	DNA	Protein	Designation	Accession No.	Designation	Accession No.
TNFR1-IgG	1	2				
TNFR2-IgG	3	4				
TNFR1-TNFR1-IgG	5	6	pTR111g-Top10'	KCCM 10288	TR111g-CHO	KCLRF-BP-00046
TNFR2-TNFR2-IgG	7	8	pTR221g-Top10'	KCCM 10291	TR221g-CHO	KCLRF-BP-00049
mgTNFR1-TNFR1-IgG	9	10	pTR111g-MG	KCCM 10404		
mgTNFR2-TNFR2-IgG	11	12	PTR221g-MG	KCCM 10407		
CD2-IgG	13	14				
CTLA4-IgG	15	16				
CD2-CD2-IgG	17	18	pCD221g	KCCM 10402		
CTLA4-CTLA4-IgG	19	20	pCT441g	KCCM 10400		
mgCD2-CD2-IgG	21	22	pCD221g-MG	KCCM 10401		
mgCTLA4-CTLA4-IgG	23	24	pCT441g-MG	KCCM 10399		

TABLE 9
Information for primers

Primer name	SEQ ID No.	Description
Oligo TNFR-EDF-EcoRI	25	Containing 5' end of the extracellular domain of TNFR1 and an EcoRI site
Oligo TNFR-EDR-IgGh	26	Reverse primer containing 3' end of the extracellular domain of TNFR1 and the hinge region of IgG
Oligo IgG1-T1F	27	Containing 5' end of the hinge region of IgG and 3' end of TNFR1
Oligo IgG1-R-XbaI	28	Reverse primer containing 3' end of the hinge region of IgG and a XbaI site
Oligo TNFR2-EDF-EcoRI	29	Containing 5' end of the extracellular domain of TNFR2 and an EcoRI site
Oligo TNFR2-EDR-IgGh	30	Reverse primer containing 3' end of the extracellular domain of TNFR2 and the hinge region of IgG
Oligo IgG1-T2F	31	Containing 5' end of the hinge region of IgG and 3' end of TNFR2
Oligo TNFR1-CF-BamHI	32	Containing 5' end of the extracellular domain of TNFR1 and a BamHI site; and used for preparation of a concatamer
Oligo TNFR1-NR-BamHI	33	Reverse primer containing 3' end of the extracellular domain of TNFR1 and a BamHI site; and used for preparation of a concatamer
Oligo TNFR2-CF-BamHI	34	Containing 5' end of the extracellular domain of TNFR2 and a BamHI site; and used for preparation of a concatamer
Oligo TNFR2-NR-BamHI	35	Reverse primer containing 3' end of the extracellular domain of TNFR2 and a BamHI site; and used for preparation of a concatamer
Oligo mgTNFR1-TNFR1-IgG-F	36	Primer for mutagenesis, containing a sequence capable of inserting glycosylation sites into the joint region of TNFR1-TNFR1, and sequences corresponding to 3' end and 5' end of TNFR1; and used for preparation of a MG (multiglycosylation) form
Oligo mgTNFR1-TNFR1-IgG-R	37	Reverse primer for mutagenesis, containing a sequence capable of inserting glycosylation sites into the joint region of TNFR1-TNFR1, and sequences corresponding to 3' end and 5' end of TNFR1; and used for preparation of a MG form
Oligo mgTNFR2-TNFR2-IgG-F	38	Primer for mutagenesis, containing a sequence capable of inserting glycosylation sites into the joint region of TNFR2-TNFR2, and sequences corresponding to 3' end and 5' end of TNFR2; and used for preparation of a MG form
Oligo mgTNFR2-TNFR2-IgG-R	39	Reverse primer for mutation, containing a sequence capable of inserting glycosylation sites into the joint region of TNFR2-TNFR2, and sequences corresponding to 3' end and 5' end of TNFR2; and used for preparation of a MG form
Oligo CD2F-EcoRI	40	Containing 5' end of the extracellular domain of CD2 and a EcoRI site
Oligo CD2R-RstI	41	Containing 3' end of the extracellular domain of CD2 and a PstI site
Oligo IgG-F-PstI	42	Containing 5' end of the hinge region of IgG and a PstI site
Oligo CTLA4F-EcoRI	43	Containing 5' end of the extracellular domain of CTLA-4 and a EcoRI site
Oligo CTLA4R-PstI	44	Containing 3' end of the extracellular domain of CTLA-4 and a PstI site
Oligo CD2-NT-F	45	Containing 5' end of the extracellular domain of CD2; and used for preparation of a concatamer
Oligo CD2-CT-R	46	Reverse primer containing 3' end of the extracellular domain of CD2; and used for preparation of a concatamer
Oligo CTLA4-NT-F	47	Containing 5' end of the extracellular domain of CTLA-4; and used for preparation of a concatamer
Oligo CTLA4-CT-R	48	Reverse primer containing 3' end of the extracellular domain of CTLA-4; and used for preparation of a concatamer
Oligo mgCD2-CD2-IgG-F	49	Used for preparation of a MG (multiglycosylation) form of CD2-CD2-IgG
Oligo mgCD2-CD2-IgG-R	50	Reverse primer used for preparation of a MG (multiglycosylation) form of CD2-CD2-IgG
Oligo mgCTLA4-CTLA4-IgG-F	51	Used for preparation of a MG (multiglycosylation) form of CTLA4-CTLA4-IgG
Oligo mgCTLA4-CTLA4-IgG-R	52	Reverse primer used for preparation of a MG (multiglycosylation) form of CTLA4-CTLA4-IgG

EXAMPLE 1**Human TNFR**

A. Manufacture of a DNA construct encoding simple fusion monomeric protein of
5 TNFR1/Fc (Fig. 1 and Fig. 5)

a. DNA fragment encoding soluble extracellular domain of TNFR1

A fusion gene encoding soluble extracellular domain of type I human TNF
receptor (TNFR1, p55) and Fc fragment of human immunoglobulin G1 was constructed by
10 the Polymerase Chain Reaction (PCR) method described in the prior art (Holten et al.,
Biotechniques 8:528, 1990).

A DNA fragment encoding soluble extracellular domain of TNFR1 was
constructed by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 25) with
EcoRI restriction site and the sequence encoding leader sequence (the sequence of amino
15 acids 1-20 of SEQ ID NO: 2), and an antisense primer (the sequence of nucleotide of SEQ
ID NO: 26) with the sequence encoding a part of 3' ends of the said soluble extracellular
domain of TNFR1 (TNFR1-ED) and 5' ends of the hinge region of immunoglobulin G1
(IgG1). The template cDNA for this reaction was constructed by reverse transcription PCR
(RT-PCR) of mRNA extracted from monocyte (T lymphocyte) of healthy adults.

20 After blood of healthy adults was extracted and diluted to 1:1 with RPMI-1640
(Gibco BRL, USA), the layer of T lymphocyte which formed at upper part was obtained by
density gradient centrifugation using Ficoll-hypaque (Amersham, USA). In order to make
the concentration of the cell to 5×10^5 cells/ml, the cell was washed with RPMI-1640 for 3
times, and RPMI-1640 culture media containing 10% Fetal Bovine Serum (FBS, Gibco
25 BRL, USA) was added, then cultured at 37°C for two days in the 5% CO₂ incubator after
adding leucoagglutinin to 3.5ug/ml (Pharmacia, USA).

The mRNAs were purified using Tri-Reagent (MRC, USA) mRNA purification kit. First, 2×10^7 of human T lymphocyte was washed with Phosphate Buffered Saline (PBS, pH7.2) for 3 times, and then 1ml of Tri-Reagent was mixed for several times to dissolve RNA. After adding 0.2ml of chloroform to this tube and mixing thoroughly, this tube was incubated at room temperature (RT) for 15 min, then centrifuged at 15,000 rpm, 4°C for 15 min. The upper part of the solution was transferred to a 1.5ml tube, and 0.5ml of isopropanol was added, and then centrifuged at 15,000 rpm, 4°C for 15 min. After the supernatant was discarded, the pellet was resuspended with 1ml of 3° distilled water treated with 75% ethanol-25% DEPC (Sigma, USA), and then centrifuged at 15,000 rpm, 4°C for 15 min. After the supernatant was removed completely and dried in the air to remove ethanol residue, RNA was resuspended with 50µl of 3° distilled water treated with DEPC.

The primary cDNA was synthesized by mixing 2µg of purified mRNA and 1µl of oligo dT (dT30, Promega, USA) primer to 10µM in 1.5ml tube, heating at 70°C for 2 min, and cooling in ice for 2 min. After that, this mixture was added with 200U of M-MLV reverse transcriptase (Promega, USA), 10µl of 5 x reaction buffer (250mM Tris-HCl, pH 8.3, 375mM KCl, 15mM MgCl₂, and 50mM DTT), 1µl of dNTP (10mM each, Takara, Japan), and DEPC-treated 3° distilled water to 50µl, then reacted at 42°C for 1 hour.

b. DNA fragment encoding Fc fragment of immunoglobulin

A DNA fragment encoding Fc fragment of immunoglobulin G1 was constructed by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 27) with the sequence encoding a part of 3' ends of the said soluble extracellular domain of TNFR and 5' end of the hinge region of immunoglobulin G1 (IgG1), and an antisense primer (the sequence of nucleotide of SEQ ID NO: 28) with XbaI restriction site and the sequence encoding 3' ends of IgG1 Fc. The template cDNA for this reaction was constructed by RT-PCR of mRNA extracted from peripheral blood cell (B lymphocyte) of convalescent patients with pyrexia of unknown origin.

c. DNA construct encoding simple fusion monomeric protein of TNFR1/Fc

After DNA fragment encoding soluble extracellular domain of TNFR1 and DNA fragment encoding Fc fragment of immunoglobulin produced as described above were mixed in the same tube, complementary binding between the common sequence (the sequence including 3' end of soluble extracellular domain of TNFR1 and 5' end of IgG1 hinge region) was induced. Using this mixture as a template, DNA construct including DNA fragment encoding soluble extracellular domain of TNFR1 and DNA fragment encoding IgG1 Fc fragment was amplified by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 25) with the sequence encoding 5' end of TNFR1 and another primer (the sequence of nucleotide of SEQ ID NO: 28) with the sequence encoding 3' end of IgG1 Fc. The constructed gene included a leader sequence to facilitate secretion of protein after expression.

d. Cloning of the DNA construct encoding simple fusion monomeric protein of TNFR1/Fc

DNA construct encoding simple fusion monomeric protein of TNFR1/Fc as described above was restricted with EcoRI and XbaI, and cloned by inserting into a commercially available cloning vector, pBluescript KS II (+) (Stratagene, USA), at EcoRI/XbaI site. The sequence of a total coding region was identified by DNA sequencing (SEQ ID NO: 1). This produced fusion protein was designated TNFR1/Fc as simple fusion monomeric protein, and the elliptical shape shown in Figure 1 represents the structure of a primary expression product of the fusion gene. The deduced amino acid sequence of simple fusion monomeric of TNFR1/Fc corresponded to SEQ ID NO: 2.

B. Manufacture of a DNA construct encoding simple fusion monomeric protein of TNFR2/Fc (Fig. 1 and Fig. 5)

a. DNA fragment encoding soluble extracellular domain of TNFR2

A fusion gene encoding soluble extracellular domain of type II human TNF receptor (TNFR2, p75) and Fc fragment of human immunoglobulin G1 was constructed by the same method as that of TNFR1/Fc.

A DNA fragment encoding soluble extracellular domain of TNFR2 was constructed by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 29) with EcoRI restriction site and the sequence encoding leader sequence (the sequence of amino acids 1-22 of SEQ ID NO: 4), and an antisense primer (the sequence of nucleotide of SEQ ID NO: 30) with the sequence encoding a part of 3' ends of said soluble extracellular domain of TNFR2 (TNFR2-ED) and 5' ends of the hinge region of immunoglobulin G1 (IgG1). The template cDNA for this reaction was constructed by RT-PCR of mRNA extracted from monocyte (T lymphocyte) of healthy adults.

b. DNA construct encoding simple fusion monomeric protein of TNFR2/Fc

After DNA fragment encoding soluble extracellular domain of TNFR2 and DNA fragment encoding Fc fragment of immunoglobulin G1 produced as described above were mixed in the same tube, complementary binding between the common sequence (the sequence including 3' end of soluble extracellular domain of TNFR2 and 5' end of IgG1 hinge region) was induced. Using this mixture as a template, DNA construct including DNA fragment encoding soluble extracellular domain of TNFR2 and encoding and DNA fragment encoding IgG1 Fc fragment was amplified by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 29) with the sequence encoding 5' end of TNFR2 and another primer (the sequence of nucleotide of SEQ ID NO: 28) with the sequence encoding 3' end of IgG1 Fc. The constructed gene includes a sequence to facilitate secretion of protein after expression.

c. Cloning of the DNA construct encoding simple fusion monomeric protein of TNFR2/Fc

DNA construct encoding simple fusion monomeric protein of TNFR2/Fc as described above was restricted with EcoRI and XbaI, and cloned by inserting into a commercially available cloning vector, pBluescript KS II (+) (Stratagene, USA), at EcoRI/XbaI site. The sequence of a total coding region was identified by DNA sequencing (SEQ ID NO: 3). This produced fusion protein was designated TNFR2/Fc as simple fusion monomeric protein, and the elliptical shape shown in Figure 1 represents the structure of a primary expression product of the fusion gene. The deduced amino acid sequence of simple fusion monomeric of TNFR2/Fc corresponded to SEQ ID NO: 4.

C. Manufacture of a DNA construct encoding concatameric fusion monomeric protein of TNFR1-TNFR1/Fc (Fig. 2 and Fig. 5)

In order to manufacture a fusion gene comprising the concatameric shape in soluble extracellular domain of TNFR1, i.e. the DNA construct encoding concatameric fusion monomeric protein of TNFR1-TNFR1/Fc, BamHI restriction site was inserted respectively into the sequence of soluble extracellular domain of TNFR1 and DNA construct as produced as above encoding simple fusion monomeric protein of TNFR1/Fc by PCR, and then regions of each fragments restricted by BamHI were linked by ligase. The DNA construct, encoding simple fusion monomeric protein of TNFR1/Fc produced as above, was used as the template of this reaction.

The fragment of the soluble extracellular domain of TNFR1 with BamHI restriction site at 3' end was amplified by PCR using a primer corresponding to the nucleotide of SEQ ID NO: 25 and another primer corresponding to the nucleotide sequence of SEQ ID NO: 33, and the other fragment of simple fusion monomeric protein of TNFR1/Fc with BamHI restriction site at 5' end was amplified by PCR using a primer

corresponding to the nucleotide of SEQ ID NO: 28 and another primer corresponding to the nucleotide sequence of SEQ ID NO: 32, respectively. PCR was performed by adding 1µl of primary cDNA, 2U of Pfu DNA polymerase (Stratagene, USA), 10µl of 10X reaction buffer [200mM Tris-HCl, pH 8.75, 100mM (NH₄)₂SO₄, 100mM KCl, 20mM MgCl₂], 1% TritonTM X-100, 1mg/ml BSA, 3µl primer 1 (10µM), 3µl primer 2 (10µM), 2µl dNTP (10mM each), and 3° distilled water to 100µl. The reaction condition was as follows; 94°C, 5 min; 95°C, 1 min; 58°C, 1 min 30 sec; 72°C, 1 min for 31 cycles; and 72°C, 15 min to make PCR product with complete blunt end.

After electrophorized on 0.8% agarose gel, the PCR product was purified by Qiaex II gel extraction kit (Qiagen, USA). The purified PCR product was restricted by BamHI and extracted by phenol-chloroform extraction methods. Subsequently, two kinds of DNA fragments restricted by BamHI were linked by ligase.

D. Manufacture of a DNA construct encoding concatameric fusion monomeric protein of TNFR2-TNFR2/Fc (Fig. 2 and Fig. 5)

After a BamHI restriction site was inserted respectively into the sequence of the soluble extracellular domain of TNFR21 and the DNA construct produced as described above encoding simple fusion monomeric protein of TNFR2/Fc by PCR, a DNA construct encoding concatameric fusion monomeric protein of TNFR2-TNFR2/Fc was manufactured by linking the regions of each fragments restricted by BamHI by ligase.

A fragment of soluble extracellular domain of TNFR2 with BamHI restriction site at 3' end was amplified using a primer corresponding the sequence of SEQ ID NO: 34 and SEQ ID NO: 35. PCR was performed as that of TNFR1, except that a DNA construct encoding simple fusion monomeric protein of SEQ ID NO: 3 produced as above was used as a template. The PCR product was purified by the method as that of TNFR1.

E. DNA construct encoding concatameric fusion monomeric protein of TNFR1-TNFR1/Fc with glycosylation motif.

5 A DNA fragment was manufactured by PCR using an antisense primer (the sequence of nucleotide of SEQ ID NO: 37) with the sequence encoding the part (the sequence of nucleotide 565-591 of SEQ ID NO: 5) of 3' end of the first soluble extracellular domain of TNFR1, except the sequence of hydrophobic peptide region (the sequence of amino acid 197-216 of SEQ ID NO: 6) at the junction of soluble extracellular domain of TNFR1 and the part (the sequence of nucleotide 649-681 of SEQ ID NO: 5) of 5' end of the
10 second soluble extracellular domain of TNFR1, and another primer (the sequence of nucleotide of SEQ ID NO: 25) with the sequence encoding EcoRI restriction site and leader sequence.

In addition, the total four amino acid sequences encoding glycosylation site (the sequence of amino acids 189-191, 192-194, 198-200, and 204-206 of SEQ ID NO: 10)
15 were inserted by manufacturing the primer as above (the sequence of nucleotide of SEQ ID NO: 36 and 37) corresponding the substitution of the nucleotide 565-567 (CTG, Leu), 574-576 (ACG, Thr), 652-654 (CTA, Leu), and 670-672 (AGA, Arg) of SEQ ID NO: 5 with the nucleotide of AAC (Asn, N); the nucleotide of 571-573 (TGC, Cys) and 580-582 (TTG, Leu) of SEQ ID NO: 5 with the nucleotide of ACC (Thr, T); the nucleotide of 658-660
20 (GAC, Asp) with the nucleotide of TCC (Ser, S).

In this reaction, the gene (the nucleotide of SEQ ID NO: 5) encoding concatameric shape of TNFR1-TNFR1/Fc was used as a template. During the primary PCR, only the half of the antisense primer was induced to bind the gene encoding concatameric shape of TNFR1-TNFR1/Fc used as a template, and, as chain reaction was
25 proceeding, the unbound part to the template was induced to form a complete double-stranded DNA by polymerase, and then this was capable of producing the DNA fragment with state of linkage of the sequence of 5' end encoding the part of the second soluble

extracellular domain and the sequence of 3' end encoding TNFR1 extracellular domain including leader sequence. Therefore, a part of the sequence of 5' end encoding the second soluble extracellular domain has the function that was capable of binding to the second DNA fragment as follows.

5 The second DNA fragment was manufactured by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 36) with the sequence encoding the part (the sequence of nucleotide 565-591 of SEQ ID NO: 5) of 3' end of the first soluble extracellular domain of TNFR1 and the part (the sequence of nucleotide 649-681 of SEQ ID NO: 5) of 5' end of the second soluble extracellular domain of TNFR1, and an antisense primer (the
10 sequence of nucleotide of SEQ ID NO: 28) with the sequence encoding a XbaI restriction site and 3' end of IgG1 Fc. This reaction was also performed as described above, that is, only the half of antisense primer was induced to bind the template, and consequently, DNA fragment like that described above had the sequence encoding 5' end of TNFR1 extracellular including the part of 3' end of the first soluble extracellular domain.

15 Subsequently, resulting from two kinds of DNA fragments as PCR described as above were mixed in the same tube, induced to bind between common sequences, and fused by PCR using primers (the sequence of nucleotide of SEQ ID NO: 25 and 28) encoding 5' and 3' end of each concatameric genes, and the product was designated mgTNFR1-TNFR1-IgG.

20 F. DNA construct encoding concatameric fusion monomeric protein of TNFR2-TNFR2/Fc with glycosylation motif.

25 A DNA fragment was manufactured by PCR using an antisense primer (the sequence of nucleotide of SEQ ID NO: 39) with the sequence encoding the part (the sequence of nucleotide 586-606 of SEQ ID NO: 7) of 3' end of first soluble extracellular domain of TNFR2, except the sequence of hydrophobic peptide region (the sequence of

amino acid 203-263 of SEQ ID NO: 8) at the junction of soluble extracellular domain of TNFR2 and the part (the sequence of nucleotide 790-807 of SEQ ID NO: 7) of 5' end of second soluble extracellular domain of TNFR2, and another primer (the sequence of nucleotide of SEQ ID NO: 29) with the sequence encoding EcoRI restriction site and leader sequence.

In addition, the total two amino acid sequences encoding glycosylation site (the sequence of amino acids 199-201 and 206-208 of SEQ ID NO: 12) were inserted by manufacturing the primer as described above (the sequence of nucleotide of SEQ ID NO: 38 and 39) corresponding to the substitution of the nucleotide 595-597 (GTC, Val) and 799-801 (GGG, Gly) SEQ ID NO: 7 with the nucleotide of AAC (Asn, N).

In this reaction, the gene (the nucleotide of SEQ ID NO: 7) encoding concatameric shape of TNFR2-TNFR2/Fc was used as a template. During the primary PCR, only the half of antisense primer was induced to bind the gene encoding concatameric shape of TNFR2-TNFR2/Fc used as a template, and, as the chain reaction was proceeding, the unbound part to the template was induced to form a complete double-stranded DNA by polymerase, and thus this was capable of producing the DNA fragment with a state of linkage of the sequence of 5' end encoding the part of the second soluble extracellular domain and the sequence of 3' end encoding TNFR2 extracellular domain including the leader sequence. Therefore, a part of the sequence of 5' end encoding the second soluble extracellular domain has the function that was capable of binding to the second DNA fragment as follows.

The second DNA fragment was manufactured by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 38) with the sequence encoding the part (the sequence of nucleotide 586-606 of SEQ ID NO: 7) of 3' end of the first soluble extracellular domain of TNFR2 and the part (the sequence of nucleotide 790-807 of SEQ ID NO: 7) of 5' end of the second soluble extracellular domain of TNFR2, and an antisense primer (the sequence of nucleotide of SEQ ID NO: 28) with the sequence encoding a XbaI restriction

site and 3' end of IgG1 Fc. This reaction was also performed, that is, only the half of antisense primer was induced to bind the template, and consequently, DNA fragment like that described above had the sequence encoding 5' end of TNFR2 extracellular including the part of 3' end of first soluble extracellular domain.

Subsequently, resulting from two kinds of DNA fragments as PCR produced as above were mixed in the same tube, induced to bind between common sequences, and fused by PCR using primers (the sequence of nucleotide of SEQ ID NO: 29 and 28) encoding 5' and 3' end of each concatameric genes, and the product was designated mgTNFR2-TNFR2-IgG.

G. Cloning of DNA constructs encoding concatameric fusion monomeric protein of TNFR-TNFR/Fc and their glycosylated forms

DNA constructs encoding concatameric fusion monomeric protein of TNFR-TNFR/Fc and their glycosylated forms as above were cloned by inserting into pBluescript KS II (+) (Stratagene, USA) at EcoRI/XbaI site. These produced fusion proteins were designated TNFR1-TNFR1/Fc and TNFR2-TNFR2/Fc as concatameric fusion monomeric protein, and designated mgTNFR1-TNFR1/Fc and mgTNFR2-TNFR2/Fc as their glycosylated forms. The deduced amino acid sequences corresponded to SEQ ID NO: 6, 8, 10, and 12.

After 10µg of pBluescript KS II (+) (Stratagene, USA) used as a vector was mixed with 15U of EcoRI, 15U of XbaI, 5µl of 10X reaction buffer (100mM Tris-HCl, pH 7.5, 100mM MgCl₂, 10mM DTT, 500mM NaCl), 5µl of 0.1% BSA (Takara, Japan), and 3rd distilled water to 50µl, DNA was restricted by incubation at 37°C for 2 hrs. After electrophorized on 0.8% agarose gel, the PCR product was purified by Qiaex II gel extraction kit (Qiagen, USA).

After 100ng of pBluescript KS II (+) (Stratagene, USA) restricted by EcoRI and XbaI was mixed with 20ng of PCR product restricted by the restriction enzyme, 0.5U of T4 DNA ligase (Amersham, USA), 1µl of 10X reaction buffer (300mM Tris-HCl, pH 7.8, 100mM MgCl₂, 100mM DTT, 10mM ATP) and 3° distilled water were added to 10µl, and the mixture was incubated in the water bath at 16°C for 16 hrs. E. coli Top10 (Novex, USA) was made to competent cell by the method of rubidium chloride (RbCl, Sigma, USA) and transformed, then spread on the solid LB media including 50µg/ml of ampicillin (Sigma, USA) and incubated at 37°C for 16 hrs. Formed colonies were inoculated in 4ml of liquid LB media including 50µg/ml of ampicillin and incubated at 37°C for 16 hrs. Plasmid was purified by the method of alkaline lysis according to Sambrook et al. (Molecular cloning, Cold Spring Harbor Laboratory press, p1.25-1.31, p1.63-1.69, p7.26-7.29, 1989) from 1.5ml of that, and the existence of cloning was confirmed by the restriction of EcoRI and XbaI.

The sequence of a total coding region was identified by the DNA sequencing method of dideoxy chain termination method (Sanger et al., Proc. Natl. Acad. Sci., 74:5483, 1977) as follows. The DNA sequencing reaction was performed according to the manual using a plasmid purified by alkaline lysis method as described above and Sequenase™ ver 2.0 (Amersham, USA). After the reaction mixture as above was loaded on 6% polyacrylamide gel and electrophorized for 2 hrs at constant voltage of 1,800-2,000 V and 50°C, DNA sequence was identified by exposing to X-ray film (Kodak, USA) after the gel was dried out.

EXAMPLE 2 AND 3

CD2 and CTLA4

DNA fragments encoding soluble extracellular domain of CD2 and CTLA4 were constructed by PCR using a primer [CD2(the sequence of nucleotide of SEQ ID NO:

40), and CTLA4(the sequence of nucleotide of SEQ ID NO: 43)] with EcoRI restriction site and the coding sequence [CD2 (the sequence of nucleotide of SEQ ID NO: 13), and CTLA4 (the sequence of nucleotide of SEQ ID NO: 15)] encoding the leader sequence [CD2(the sequence of amino acid 1-24 of SEQ ID NO: 14), and CTLA4(the sequence of amino acid 1-21 of SEQ ID NO: 16)], and an antisense primer [CD2(the sequence of nucleotide of SEQ ID NO: 41), and CTLA4(the sequence of nucleotide of SEQ ID NO: 44)] with PstI restriction site and the sequence [CD2(the sequence of nucleotide of SEQ ID NO: 13), and CTLA4(the sequence of nucleotide of SEQ ID NO: 15)] encoding 3' end of the soluble extracellular domain of the proteins as described above. The template cDNA for this reaction was constructed by reverse transcription PCR (RT-PCR) of mRNA extracted from the monocyte (T lymphocyte) of healthy adults.

Also, a DNA fragment encoding Fc fragment of immunoglobulin G1 was constructed by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 42) with PstI restriction site and the sequence encoding 5' ends of constant region of IgG1, and an antisense primer (the sequence of nucleotide of SEQ ID NO: 28) with XbaI restriction site and the sequence encoding 3' ends of IgG1 Fc. The template cDNA for this reaction was constructed by RT-PCR of mRNA extracted from peripheral blood cell (B lymphocyte) of convalescent patients with unknown fever.

Subsequently, both DNA fragment encoding soluble extracellular domain of CD2 and CTLA4 and DNA fragment encoding Fc fragment of immunoglobulin G1 produced as described above were restricted by PstI, and then the simple dimeric shape of CD2/Fc and CTLA4/Fc genes were constructed by linkages using T4 DNA ligase. The constructed genes included a leader sequence to facilitate secretion of protein after expression.

DNA constructs as described above were restricted by restriction enzyme of EcoRI and XbaI, and cloned by inserting into a commercially available cloning vector, pBluescript KS II (+) (Stratagene, USA) at EcoRI/XbaI site. The sequence of a total coding

region was identified by DNA sequencing (SEQ ID NO: 13 and 15). These produced fusion proteins were designated CD2/Fc and CTLA4/Fc, and the deduced amino acid sequences of these corresponded to SEQ ID NO: 14 and 16.

PCR was performed by adding 1 μ l of primary cDNA, 2U of Pfu DNA polymerase (Stratagene, USA), 10 μ l of 10X reaction buffer [200mM Tris-HCl, pH 8.75, 100mM (NH₄)₂SO₄, 100mM KCl, 20mM MgCl₂], 1% TritonTM X-100, 1mg/ml BSA, 3 μ l primer 1 (10 μ M), 3 μ l primer 2 (10 μ M), 2 μ l dNTP (10mM each), and 3° distilled water to 100 μ l. The reaction condition was as follows; 94°C, 5 min; 95°C, 1 min; 58°C, 1 min 30 sec; 72°C, 1 min for 31 cycles; and 72°C, 15 min to make PCR product with complete blunt end.

The fusion genes with concatameric shape of CD2-CD2/Fc and CTLA4-CTLA4/Fc were constructed as follows.

In order to manufacture fusion gene comprising the concatameric shape in soluble extracellular domain of CD2 and CTLA4, the sequences of soluble extracellular domain of CD2 and CTLA4 were inserted by blunt-end ligation using ligase at the junction between extracellular domain and immunoglobulin of fusion genes in the shape of simple dimer with blunt end, using PstI restriction enzyme and T4 DNA polymerase. Specifically, DNA constructs were constructed by PCR using a primer [CD2(the sequence of nucleotide of SEQ ID NO: 13) and CTLA4(the sequence of nucleotide of SEQ ID NO: 48)] with the coding sequence [CD2(the sequence of nucleotide of SEQ ID NO: 13) and CTLA4(the sequence of nucleotide of SEQ ID NO: 15)] encoding the end of leader sequence [CD2(the sequence of amino acid 25 of SEQ ID NO: 14) and CTLA4(the sequence of amino acid 22 of SEQ ID NO: 16)] of soluble extracellular domain, and an antisense primer [CD2(SEQ ID NO: 46) and CTLA4(SEQ ID NO: 48)] with the sequence [CD2(the sequence of nucleotide of SEQ ID NO: 13) and CTLA4(the sequence of nucleotide of SEQ ID NO: 15)] encoding 3' end of soluble extracellular domain as above. The simple fusion monomeric genes [CD2/Fc (the sequence of nucleotide of SEQ ID NO: 13) and CTLA4/Fc (the sequence of

nucleotide of SEQ ID NO: 15)] described as above were used as the template of this reaction.

Also, CD2/Fc and CTLA4/Fc, which were inserted in pBluescript KS II (+) in the shape of simple monomeric form, were made to have 3' overhang end using the restriction enzyme of PstI. The cut end of 3' overhang was partially deleted to form a blunt end by treating T4 DNA polymerase. In order to manufacture fusion genes in the shape of concatamer in soluble extracellular domain, the soluble extracellular domains of CD2 and CTLA4 produced by PCR as described above were cloned by inserting into cut ends of simple monomeric gene made as blunt end. These produced fusion proteins were designated CD2-CD2/Fc and CTLA4-CTLA4/Fc as concatameric fusion monomeric protein, and their deduced amino acid sequences corresponded SEQ ID NO: 18 and 20, respectively.

The concatameric fusion genes in the shape of multiglycosylated form were constructed as follows.

The glycosylation motif was inserted by secondary PCR with mixing in the same tube of a DNA fragment produced by PCR using a primer including EcoRI restriction site and the soluble extracellular domain with leader sequence, and an antisense primer with the sequence encoding the part of 3' end of the first soluble extracellular domain of concatameric shape of fusion gene and the part of 5' end of the second soluble extracellular domain with the nucleotide of substituted glycosylation motif; and other DNA fragment produced by PCR using a primer with the sequence encoding the part of 3' end of the first soluble extracellular domain of concatameric shape of fusion gene and the part of 5' end of the second soluble extracellular domain with the nucleotide of substituted glycosylation motif, and an antisense primer with the sequence encoding 3' end of Fc fragment of immunoglobulin G1 and XbaI restriction site.

In the case of concatameric fusion gene of CD2/Fc and CTLA4/Fc, the glycosylation motif was inserted by PCR using modified primers as the same methods as

that of TNFR/Fc described as above, but it was different from the case of TNFR/Fc that the amino acid sequence of binding to soluble extracellular domain of CD2 and CTLA4 was retained as the same.

In the process of multiglycosylation of the concatameric fusion protein of CD2/Fc and CTLA4/Fc, the case of CD2/Fc was completed by inserting the total two glycosylation motif peptide region (the sequence of amino acid of 200-202 and 206-208 of SEQ ID NO: 22) using a manufactured primer including the substitution of the nucleotide of 598-600 (CCT, Pro) and 616-618 (GAG, Glu) of SEQ ID NO: 17 with AAT (Asn, N), and the case of CTLA4/Fc was completed by inserting the total three glycosylation motif peptide region (the sequence of amino acid of 136-138, 142-144, and 147-149 of SEQ ID NO: 24) using a manufactured primer (SEQ ID NO: 51 and 52) including the substitution of the nucleotide of 403-405 (GTA, Val) and 424-426 (CCA, Pro) of SEQ ID NO: 19 with AAT (Asn, N); the nucleotide of 409-411 (GAT, Asp) and 445-447 (GTG, Val) with ACA (Thr, T) and ACG (Thr, T), respectively. These produced fusion proteins were designated mgCD2-CD2/Fc and mgCTLA4-CTLA4/Fc as concatameric fusion monomeric protein, and their deduced amino acid sequences corresponded to SEQ ID NO: 22 and 24, respectively.

EXAMPLE 4

Expression and purification of simple/concatameric fusion dimeric protein of TNFR/Fc

In order to express the fusion proteins in CHO-K1 cell (ATCC CCL-61, Ovary, Chinese hamster, *Cricetulus griseus*), after pBluescript KS II (+) plasmid DNA including TNFR/Fc fusion gene was purified from transformed *E. coli*, an animal cell expression vectors were constructed as TNFR/Fc fragment produced by restriction using EcoRI and XbaI was inserted at EcoRI/XbaI site of an animal cell expression vector, pCRTM3

(Invitrogen, USA) plasmid. And these were designated plasmid pTR11-Top10' and plasmid pTR22-Top10', and deposited as accession numbers of KCCM 10288 and KCCM 10291, respectively, at Korean Culture Center of Microorganisms (KCCM) on Jul. 10. 2001.

5 Transfection was performed by mixing either the plasmid pTR11-Top10' or plasmid pTR22-Top10' DNA including TNFR/Fc fusion genes as described above with the reagent of Lipofectamin™ (Gibco BRL, USA). CHO-K1 cells with the concentration of $1-3 \times 10^5$ cells/well were inoculated in 6-well tissue culture plate (Nunc, USA), and incubated to 50-80% in 10% FBS - DMEM media, then the DNA-liposome complex, which was reacted for 15-45 min with 1-2 μ g of either the plasmid pTR11-Top10' or
10 plasmid pTR22-Top10' DNA including TNFR/Fc fusion genes as described above and 2-25 μ l of Lipofectamin™ (Gibco BRL, USA), were added to the cell culture plate in the serum-free DMEM media. After incubation for 5 hrs, DMEM media with 20% serum was added and cells were incubated further for 18-24 hrs. After primary transfection, cells were incubated for 3 weeks in 10% FBS - DMEM media with 1.5mg/ml of Geneticin (G418,
15 Gibco BRL, USA), and formed colonies was selected for amplified incubation. The expression of fusion proteins was analyzed by ELISA using a peroxidase labeled goat anti-human IgG (KPL, USA).

ELISA was performed as follows. First, 1mg/ml of a peroxidase labeled goat anti-human IgG (KPL, USA) was diluted to 1:2,000 with 0.1M sodium bicarbonate, 100 μ l of that was aliquoted into 96-well flexible plate (Falcon, USA) and sealed with plastic wrap, then incubated at 4°C over 16 hrs to be coated on the surface of the plate. After this, it was washed for 3 times with washing buffer (0.1% Tween-20 in 1X PBS) and dilution buffer (48.5ml 1XPBS, 1.5ml FBS, 50ul Tween-20), and then was aliquoted to 180l. After
20 20 μ l of culture supernatant was dropped in the first well, then serially diluted using a micropipette, and 0.01 μ g/ μ l of human immunoglobulin G (Sigma, USA) as the positive control and the culture media of untransfected CHO K-1 cell as the negative was equally
25 diluted. After dilution, 96-well ELISA plate (Falcon, USA) was wrapped with aluminum

foil and incubated at 37°C for 1 hr 30 min, washed for 3 times with washing buffer. Peroxidase conjugated goat anti-human IgG (KPL, USA) was diluted to 1:5,000 with dilution buffer, aliquoted to 100µl, wrapped with aluminum foil, and reacted at 37°C for 1 hr. After reaction, this plate was washed for 3 times, colorized using TMB microwell
5 peroxidase substrate system (KPL, USA) and existence of expression was confirmed by measurement of absorbance at 655nm wavelength using microplate reader (Bio-Rad, Model 550, Japan).

Transfectants manufactured as above were designated TR11Ig-CHO and TR22Ig-CHO and deposited as accession numbers of KCLRF-BP-00046 and KCLRF-BP-
10 00049, respectively, at Korean Cell Line Research Foundation (KCLRF) on Jul. 7. 2001. And adaptation for transfectants as described above to one of the serum free media, CHO-S-SFM II (Gibco BRL, USA), was proceeded to purify the proteins produced by those transfectants as follows. After about 3×10^5 of cells were inoculated into the 6-well plate, cells were cultured at 5% CO₂, 37°C for over 16 hrs to adhere, and it was checked under a
15 microscope that cells were adhered at about 30~50% area of the plate, then cells were cultured in a media consisting of 10% FBS DMEM and CHO-S-SFM II in the ratio of 8:2. After culturing 3 times serial passage at this ratio, it was cultured 3 times at the ratio of 6:4; 3 times at 4:6; 3 times at 3:7; 3 times at 2:8; 3 times at 1:9; and finally cultured in 100% CHO-S-SFM II media. And the level of expression was measured by ELISA.

20 After these transfectant cells were cultured on a large scale in CHO-S-SFM II, the supernatants including each fusion proteins were centrifuged at 200X g for 12min to remove cell debris, and proteins were purified by the method using HiTrap protein A column (Amersham, USA) as follows. After 20mM of sodium phosphate (pH 7.0, Sigma, USA) was passed at the velocity of 1ml/min for 2 min, 10ml of supernatant was passed at
25 the same velocity to bind fusion protein to protein A. After 20mM of sodium phosphate (pH 7.0) was passed at the same velocity for 2 min to wash, 500µl of the extracts were serially fractionated in a 1.5ml tube as 0.1M of citric acid (pH 3.0, Sigma, USA) was

passed at the the same velocity for 3 min. This was adjusted to pH 7.0 using 1M of Tris (pH 11.0, USB, USA), the existence of fusion proteins in tube was confirmed through ELISA as described above. The purified proteins were concentrated by centrifugation at 2000Xg, 4 °C for 30min using Centricon 30 (Amicon, USA)

Example 5.

SDS-PAGE of purified TNFR1-TNFR1/Fc and TNFR2-TNFR2/Fc (Fig. 15)

Proteins purified using protein A column were electrophorized by the method of SDS-PAGE in reducing condition added by DTT, reducing reagent (which destroy disulfide bond), and in a non-reducing condition excluding DTT. The result of the estimation of molecular weight on SDS-PAGE is shown in Table 10. It was possible to confirm that TNFR/Fc proteins were the shape of a dimer in the cell. The molecular weight deduced from the amino acid sequence of TNFR1-TNFR1-Ig was about 70kDa, and was estimated as about 102kDa on SDS-PAGE. As this difference could be regarded as a general phenomenon which generate on the electrophoresis of glycoproteins, this feature seemed to occur as the result from decrease in mobility on the electrophoresis by the site of glycosylation.

Table 10. Molecular weight of TNFR-TNFR/Fc on the SDS-PAGE.

Proteins	Molecular weight (kDa)	
	Reducing condition	Non-reducing condition
TNFR1-TNFR1/Fc	102	200
TNFR2-TNFR2/Fc	115	220

Example 6.

Experiment of neutralization effect of simple/concatameric fusion dimeric TNFR/Fc fusion proteins on the cytotoxicity of TNF α and TNF β

An L929 cell [ATCC, Mus musculus (mouse), NCTC clone 929 (derivative of strain L; L-929; L cell)] was used for testing the effect of TNFR/Fc fusion protein on the inhibition of cytotoxicity induced by TNF α and TNF β . This analysis was based on the TNFR activity of inhibiting cytotoxicity induced by TNF (Scallon et al., Cytokine 7:759, 1995).

L929 cells were inoculated to be 3×10^4 cells/well in 96-well plates, and incubated at 37°C for 24 hrs in a CO₂ incubator. Subsequently, actinomycin D (Sigma, USA) was added to 3 μ g/ml, and cells were incubated for 16~18 hrs with TNF α and TNF β in the concentration of expressing 100% cytotoxicity (0.5~2ng/ml), and with serially 10 times diluted TNFR sample. Then, the cells in the 96-well plate were stained by the staining reagent, crystal violet (Wako Pure Chemical Industries, Japan) and the activity of the cells was estimated by the degree of absorbance at 595 nm wavelength using a spectrophotometer (Bio-Rad, Model-550, Japan).

As shown in Table 11 represented by IC₅₀ of each TNFR/Fc fusion protein, concatameric fusion proteins (TNFR1-TNFR1/Ig and TNFR2-TNFR2/Ig) have shown the higher inhibitory effect on the cytotoxicity induced by two kinds of TNF than simple dimeric fusion proteins (TNFR1/Ig and TNFR2/Ig). Also, as compared with the effects of existing simple fusion dimer and concatameric shaped TNFR/Fc fusion protein dimer of the present invention on the inhibition of cytotoxicity of TNF α (Fig. 16) and TNF β (Fig. 17), it more clearly appeared that concatameric shaped TNFR/Fc fusion protein dimers of the present invention remarkably inhibited the TNF α and TNF β cytotoxicity.

Table 11. IC₅₀ of cytotoxicity inhibition

Fusion proteins	IC ₅₀ (ug/ml)
-----------------	--------------------------

		TNF α treated	TNF β treated
Simple dimer	[TNFR1/Fc] ₂	63	129
	[TNFR2/Fc] ₂	189	469
Concatameric dimer	[TNFR1-TNFR1/Fc] ₂	9	20
	[TNFR2-TNFR2/Fc] ₂	15	15

Example 7

Experiment of suppressive effect of simple/concatameric fusion dimeric CD2/Fc fusion protein and CTLA4/Fc fusion protein on the proliferation of active immune cell

WT100B1S, a cell line of B lymphocyte which was made by transfection of pyrexia patient's B lymphocyte with Epstein-Barr virus was incubated in RPMI 1640 supplemented with 10% FBS to use as antigen presenting cell of T lymphocyte. After centrifuged at 2,000rpm for 2 min to precipitate, this cells were resuspended in RPMI 1640 supplemented with 10% FBS to make 5.0×10^5 cells/ml, then irradiated by 3,000 rd of γ -ray.

T lymphocytes were isolated from blood of healthy adult using Ficoll-hypaque (Amersham, USA), then incubated RPMI 1640 supplemented with 10% FBS to 2.0×10^6 cells/ml.

To perform primary Mixed Lymphocyte Reaction (MLR), each 15ml of WT100B1S and T lymphocyte were mixed in 150mm cell culture dish, and incubated for 3 days, then added by 15ml of RPMI 1640 supplemented with 10% FBS and incubated for 3 days further. After incubated for total 6 days, live T lymphocytes were purified using Ficoll-hypaque (Amersham, USA) as described above, and purified T lymphocytes were stored in liquid nitrogen after freezing it by using the media comprising 45% FBS, 45% RPMI 1640, and 10% DMSO.

After T lymphocytes which were reacted by primary MLR were thawed to perform secondary MLR, the cells were washed with RPMI 1640 media for 2 times and made to be 3.0×10^5 cells/ml in RPMI 1640 supplemented with 10% FBS.

WT100B1S using as antigen presenting cell was newly cultured by the method as described above, then prepared by irradiation of 3,000 rd of γ -ray and to be 7.5×10^4 cells/ml in RPMI 1640 supplemented with 10% FBS. After 100 μ l of prepared WT100B1S was added in 96-well flat bottom cell culture plate and mixed with CD2/Fc and CTLA4/Fc fusion protein at final concentration of 10, 1, 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4} g/ml, 100 μ l of primary MLR reacted T lymphocytes as above was added. After incubated for 2 days in 5% CO₂, 37°C incubator, 100 μ l of RPMI 1640 supplemented with 10% FBS was added and incubated for 2 days further. In the last 6 hrs of the total 6 days culture, cells were incubated with addition of 1.2 μ Ci/ml of 3 H-thymidine (Amersham, USA).

At the end of culturing, supernatants were removed after centrifugation of 96-well plate was performed at 4°C, 110Xg for 10 min to precipitate T lymphocytes, and pellets were washed with 200 μ l of 1XPBS. Centrifugation was performed in the same condition and PBS was removed, then 200 μ l of ice-cold trichloridic acid (TCA, Merck, USA) was added and mixed for 2 min, then reacted at 4°C for 5 min to remove residue of 3 H-thymidine.

After centrifugation in the same condition as described above, supernatants were removed and T lymphocytes were fixed by incubation at 4°C for 5 min after 200 μ l of ice-cold 70% ethanol was added. Supernatants were removed after centrifugation, and 3 H-thymidine (Amersham, USA) residue was completely removed by treatment of 10% TCA in the same method as described above.

Cell lysis was performed by reaction with 100 μ l of 2% SDS (pH 8.0) and 0.5N of NaOH at 37°C for 30min, and T lymphocytes were precipitated by centrifugation at 25°C, 110Xg for 10min, and then 50 μ l of supernatants was transferred to 96-well sample plate (Wallac, USA). After 1.5 volume of OptiPhase SuperMix (Wallac, USA) was added into the supernatants and mixed for 5 min, the existence of T lymphocyte proliferation was confirmed by measurement of cpm value of 3 H using 1450 MicroBeta TriLux microplate liquid scintillation and luminescence counter (Wallac, USA).

Example 8

Experiment of effect on increase of plasma half-life of glycosylated concatameric fusion dimeric proteins in mouse

5 The measurement of plasma half-life of glycosylated concatameric fusion dimeric proteins, [mgTNFR1-TNFR1/Fc]2, [mgTNFR2-TNFR2/Fc]2, [mgCD2-CD2/Fc]2, and [mgCTLA4-CTLA4/Fc]2 was performed by measuring the concentration of proteins using ELISA after 5µg of purified fusion proteins was i.p. injected into mouse (ICR, 10 Samtako, Korea) and bloods were extracted at regular interval for 120 hrs (5 days) as maximum. As shown Fig. 20, Fig. 21, and Fig 22, it could be seen that the plasma half-life of glycosylated concatameric fusion dimeric proteins have been increased in comparison of the corresponding simple fusion dimeric proteins of native shape, and the increase in efficacy through continuous effect could be expected.

Example 9

Experiment of effects of simple/concatameric TNFR/Fc fusion protein dimers on collagen-induced arthritis of DBA/1 mouse

20 Collagen Induced Arthritis (CIA) was developed by injection with 100µg per DBA/1 mouse of type II collagen dissolved at 2mg/ml concentration in 0.05M acetic acid and Arthrogen-CIA adjuvant (Chondrex, USA) into tail. Boosting was performed after 3 weeks, and incomplete Freund's adjuvant (Difco, USA) was used.

25 Arthritis was developed 3~4 weeks after immunization with 100µg of type II collagen in the DBA/1 mice. Red and swollen paws of mice had been observed 3~5 days after onset, and inflammatory arthritis lasted more than 3 - 4 weeks. Although inflammation was eventually alleviated, damaged joints remained rigid permanently. The degree of

arthritis was measured 2-3 times per week on the basis of table 12 which represented subjective index of arthritis severity (measure average of five mice in each experiment). To measure the effects of simple and concatameric fusion dimeric TNFR/Fc on CIA, TNFR/Fc or PBS was i.p. injected into the mice. TNFR/Fc was injected with 10 μ g at every 2 days for 19-45 days into 5 mice per experiments (arrows in Fig. 23). PBS was injected into 5 mice as control. As shown in Fig. 7, in the case of mice injected with existing simple dimeric shaped TNFR/Fc fusion protein, it could be seen that the effect decreased to about 26-38% in comparison with the figures of arthritis index in mice injected with PBS as control, but 42-55% decreased in case of concatameric shaped dimer, [TNFR1-TNFR1/Fc]₂ and [TNFR2-TNFR2/Fc]₂ were injected. Therefore, it could be shown that concatameric fusion dimeric TNFR/Fc fusion proteins have remarkably decreased arthritis of mouse than existing simple fusion dimeric TNFR/Fc fusion proteins.

Table 12. Severity score of arthritis

Severity score	Condition of disease
0	No erythema and swelling
1	Erythema and mild swelling limited to ankle and tarsal
2	Erythema and mild swelling spread from ankle to tarsal
3	Erythema and mild swelling spread from ankle to metatarsal joint
4	Erythema and severe swelling extend to ankle, legs, and digits

15

The results as above represented that concatameric shaped dimeric TNFR/Fc fusion proteins were more effective in decreasing the rate of CIA development than existing simple dimeric fusion proteins, therefore, as use in arthritis therapy, concatameric shaped protein compositions could be more effective therapeutics than existing protein compositions.

20

The concatameric proteins, concatameric fusion dimeric proteins and their glycosylated proteins of the present invention were able to express increased efficacy and high stability, and to be produced with high yield.

5

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Date of deposit 29/06/2001	Accession Number KCLRF-BF-00046
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Korean Culture Center of Microorganisms(KCCM)	
Address of depositary institution (including postal code and country)	
361-221, Yurim B/D, Hongje-1-dong, Seodaemun-gu, SEOUL 120-091, Republic of Korea	
Date of deposit 11/07/2002	Accession Number KCCM 10404
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only
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Form PCT/RO/134 (July 1998)

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 29, line 15-20 .	
B. IDENTIFICATION OF DEPOSIT Further deposits are on an additional sheet <input type="checkbox"/>	
Name of depositary institution Korean Culture Center of Microorganisms(KCCM)	
Address of depositary institution(<i>including postal code and country</i>) 361-221, Yurim B/D, Hongje-1-dong, Seodaemun-gu, SEOUL 120-091, Republic of Korea	
Date of deposit 11/07/2002	Accession Number KCCM 10403
C. ADDITIONAL INDICATIONS (<i>delete this if not applicable</i>) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (<i>if the indications are not for all designated States</i>)	
E. SEPARATE FURNISHING OF INDICATIONS (<i>leave blank if not applicable</i>) The indications listed below will be submitted to the International Bureau Inter(<i>specify the general nature of the indications e.g., "Accession Number of Deposit"</i>)	

For receiving Office use only <input type="checkbox"/> This sheet was received with the international application Authorized officer	For international Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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Form PCT/RO/134(July 1998)

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 29, line <u>15-20</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are on an additional sheet <input type="checkbox"/>	
Name of depositary institution Korean Culture Center of Microorganisms(KCCM)	
Address of depositary institution (including postal code and country) 361-221, Yurim B/D, Hongje-1-dong, Seodaemun-gu, SEOUL 120-091, Republic of Korea	
Date of deposit 11/07/2002	Accession Number KCCM 10405
C. ADDITIONAL INDICATIONS <i>(leave blank if not applicable)</i> This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE <i>(if the indications are not for all designated States)</i>	
E. SEPARATE FURNISHING OF INDICATIONS <i>(leave blank if not applicable)</i>	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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WHAT IS CLAIMED IS:

1. A concatameric protein comprising two soluble domains, in which a N-terminus of a soluble domain of a biologically active protein is linked to C-terminus of an identical soluble domain or a different soluble domain of a biologically active protein.
- 5 2. A concatameric fusion dimeric protein comprising two monomeric proteins formed by linkage of a concatamer of two identical soluble extracellular domains of proteins involving immune response to a hinge region of an Fc fragment of an immunoglobulin molecule, wherein said monomeric proteins are linked by intermolecular disulfide bonds at the hinge region, and having improved stability and therapeutic effects.
- 10 3. The concatameric fusion dimeric protein as set forth in claim 2, wherein the immunoglobulin molecule is IgG.
4. The concatameric fusion dimeric protein as set forth in claim 2, wherein the protein involving immune response is selected from the group consisting of cytokines, cytokine receptors, adhesion molecules, tumor necrosis factor receptors, receptor tyrosine
15 kinases, chemokine receptors and other cell surface proteins which contain a soluble extracellular domain.
5. The concatameric fusion dimeric protein as set forth in claim 4, wherein the protein is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-17, TNF, TGF, IFN, GM-CSF, G-CSF, EPO, TPO, M-CSF, GHR, IL-13R,
20 IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, IL-15R, TNFR, TGFR, IFNR, interferon- α R, β R and γ R, GM-CSFR, G-CSFR, EPOR, cMpl, gp130, Fas (Apo 1), CCR1, CXCR1-4, TrkA, TrkB, TrkC, Htk, REK7, Rse/Tyro-3, hepatocyte growth factor R, platelet-derived growth factor R, Flt-1, CD2, CD4, CD5, CD6, CD22, CD27, CD28, CD30, CD31, CD40, CD44, CD100, CD137, CD150, LAG-3, B7, B61, β -neurexin, CTLA-4,
25 ICOS, ICAM-1, complement R-2 (CD21), IgER, lysosomal membrane gp-1, α 2-microglobulin receptor-related proteins, and sodium-releasing peptide R.

6. The concatameric fusion dimeric protein as set forth in claim 2, wherein the monomeric protein contains an amino acid sequence of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 18, or SEQ ID NO: 20.

7. A DNA construct encoding a monomeric protein formed by linkage of a concatamer of two identical soluble extracellular domains of a protein involving immune response to a hinge region of an Fc fragment of an immunoglobulin molecule.

8. The DNA construct as set forth in claim 7, wherein the immunoglobulin molecule is IgG.

9. The DNA construct as set forth in claim 7, wherein the protein involving immune response is selected from the group consisting of cytokines, cytokine receptors, adhesion molecules, tumor necrosis factor receptors, receptor tyrosine kinases, chemokine receptors and other cell surface proteins which contain a soluble extracellular domain.

10. The DNA construct as set forth in claim 9, wherein the protein is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-17, TNF, TGF, IFN, GM-CSF, G-CSF, EPO, TPO, M-CSF, GHR, IL-13R, IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, IL-15R, TNFR, TGFR, IFNR, interferon- α R, - β R and - γ R, GM-CSFR, G-CSFR, EPOR, cMpl, gp130, Fas (Apo 1), CCR1, CXCR1-4, TrkA, TrkB, TrkC, Htk, REK7, Rse/Tyro-3, hepatocyte growth factor R, platelet-derived growth factor R, Flt-1, CD2, CD4, CD5, CD6, CD22, CD27, CD28, CD30, CD31, CD40, CD44, CD100, CD137, CD150, LAG-3, B7, B61, β -neurexin, CTLA-4, ICOS, ICAM-1, complement R-2 (CD21), IgER, lysosomal membrane gp-1, α 2-microglobulin receptor-related proteins, and sodium-releasing peptide R.

11. The DNA construct as set forth in claim 7, wherein the DNA construct contains a nucleotide sequence of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 17, or SEQ ID NO: 19.

12. A recombinant expression plasmid comprising the DNA construct of claim 7 operably linked thereto.

13. The recombinant expression plasmid as set forth in claim 12, wherein the recombinant expression plasmid is a pTR11-Top10' plasmid (accession No.: KCCM 10288),
5 a pTR22-Top10' plasmid (accession No.: KCCM 10289), a pCD22Ig plasmid (accession No.: KCCM 10402), or a pCT44Ig plasmid (accession No.: KCCM 10400).

14. A host cell transformed or transfected with the recombinant expression plasmid of claim 12.

15. The host cell as set forth in claim 14, wherein the host cell is a
10 mammalian cell.

16. The host cell as set forth in claim 14 or 15, wherein the recombinant expression plasmid is a pTR11-Top10' plasmid (accession No.: KCCM 10288), a pTR22-Top10' plasmid (accession No.: KCCM 10289), a pCD22Ig plasmid (accession No.: KCCM 10402), or a pCT44Ig plasmid (accession No.: KCCM 10400).

17. The host cell as set forth in claim 16, wherein the host cell is a TR11Ig-CHO cell line (accession No.: KCLRF-BP-00046) or a TR22Ig-CHO cell line (accession
15 No.: KCLRF-BP-00049).

18. A method of preparing a concatameric fusion dimeric protein in which disulfide bonds are formed between the hinge regions of two monomeric proteins,
20 comprising the steps of:

culturing the transformed or transfected host cell of claim 14 under conditions suitable for expression of a DNA construct encoding a concatameric fusion monomeric protein in which a concatamer of two identical soluble extracellular domains of

proteins involving immune response is linked to a hinge region of an Fc fragment of an immunoglobulin molecule; and

isolating and purifying a dimeric protein formed by dimerization of the produced monomeric proteins from culture medium.

- 5 19. The method as set forth in claim 18, wherein the DNA construct encoding a concatameric fusion monomeric protein is prepared by preparing a DNA construct encoding a simple fusion monomeric protein formed by joining a DNA fragment encoding an Fc fragment of an immunoglobulin molecule and a DNA fragment encoding a soluble extracellular domain of a protein involving immune response; and joining the prepared
10 DNA construct and a second DNA fragment identical to the DNA fragment encoding a soluble extracellular domain of a protein involving immune response.

20. The method as set forth in claim 19, wherein the DNA construct encoding a concatameric fusion monomeric protein contains a glycosylation motif sequence.

- 15 21. The method as set forth in claim 20, wherein the glycosylation motif sequence is inserted to a region at which two soluble extracellular domains are joined.

22. The method as set forth in claim 19, wherein the concatameric fusion monomeric protein contains a leader sequence.

- 20 23. The method as set forth in claim 22, wherein the concatameric fusion monomeric protein is CTLA-4, and the leader sequence has an amino acid sequence of
MACLGFRHKAQKNLAARTWPCTLLFFIPVFCKA.

24. The method as set forth in claim 23, wherein the leader sequence has an amino acid sequence of MRTWPCTLLFFIPVFCKA excluding ACLGFRHKAQKNLAA.

25. The method as set forth in any of claims 18 to 24, wherein the host cell is a mammalian cell.

26. A concatameric fusion dimeric protein comprising two monomeric proteins formed by linkage of a concatamer of two identical soluble extracellular domains of proteins involving immune response to the hinge region of Fc fragment of an immunoglobulin molecule, wherein said monomeric proteins are linked by formation of intermolecular disulfide bonds at the hinge region and glycosylated, and having improved stability and therapeutic effects.

27. The concatameric fusion dimeric protein as set forth in claim 26, wherein the monomeric protein contains an amino acid sequence of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 22, or SEQ ID NO: 24.

28. A DNA construct encoding a monomeric protein formed by linkage of a concatamer of two identical soluble extracellular domains of proteins involving immune response to a hinge region of an Fc fragment of an immunoglobulin molecule and containing glycosylation motif peptides.

29. The DNA construct as set forth in claim 28, wherein the DNA construct contains an amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 21, or SEQ ID NO: 23.

30. A recombinant expression plasmid operably linked to the DNA construct of claim 28.

31. The recombinant expression plasmid as set forth in claim 30, wherein the recombinant expression plasmid is a pTR11Ig-MG plasmid (accession No.: KCCM 10404), a pTR22Ig-MG plasmid (accession No.: KCCM 10407), a pCD22Ig-MG plasmid (accession No.: KCCM 10401), or a pCT44Ig-MG plasmid (accession No.: KCCM 10399).

32. A host cell transformed or transfected with the recombinant expression plasmid of claim 30.

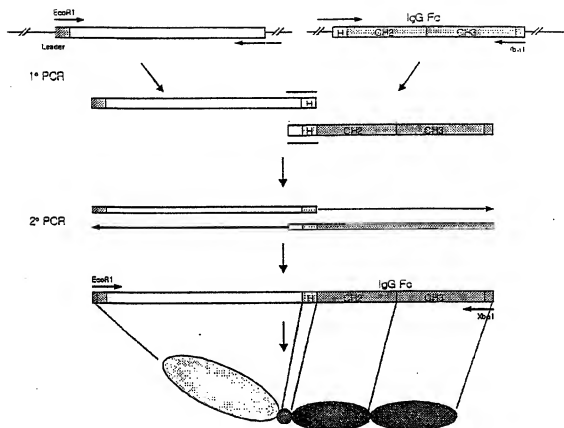
33. The host cell as set forth in claim 32, wherein the host cell is a mammalian cell.

34. A pharmaceutical or diagnostic composition comprising the dimeric protein of claim 2.

5 35. A pharmaceutical or diagnostic composition comprising the glycosylated dimeric protein of claim 26.

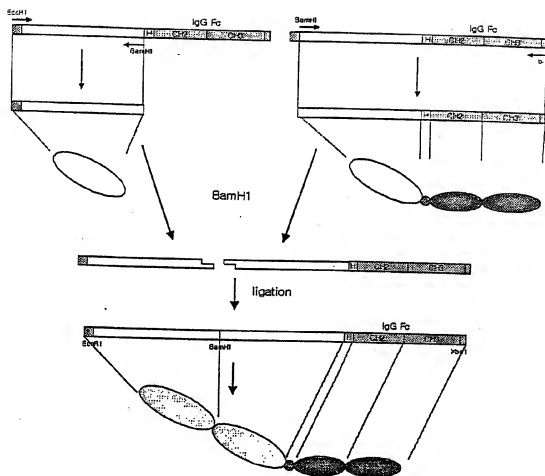
1/23

FIG. 1



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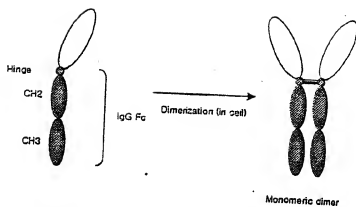
FIG. 2



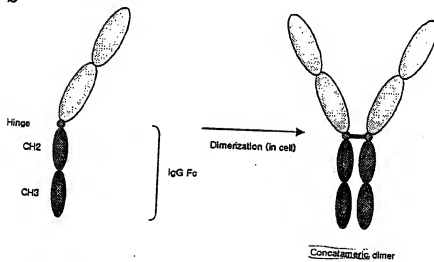
3/23

FIG. 3

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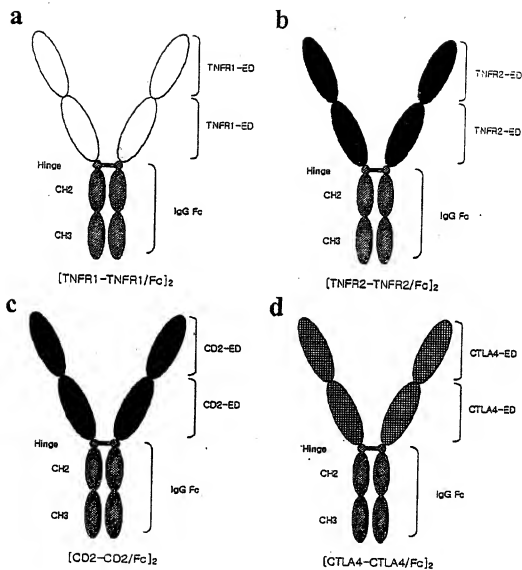


b



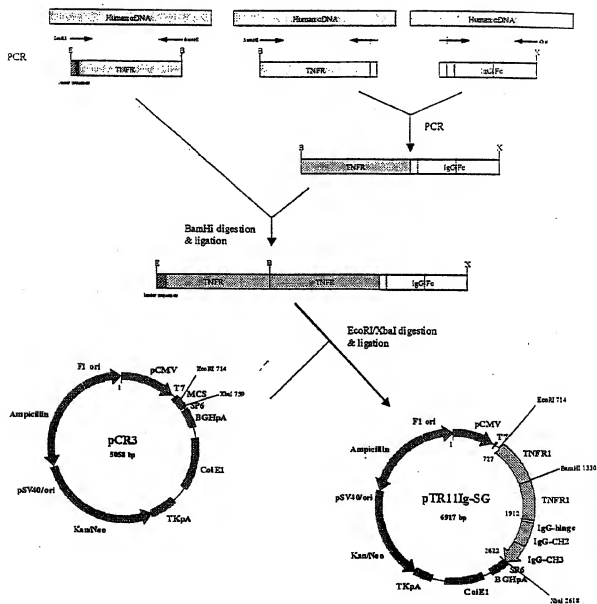
4/23

FIG. 4



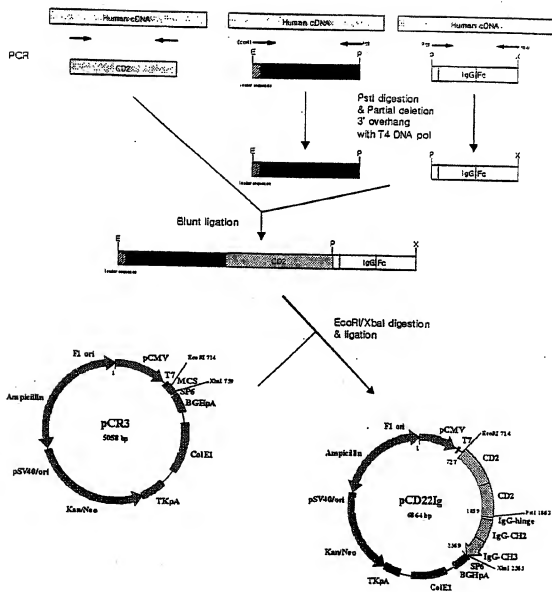
5/23

FIG. 5



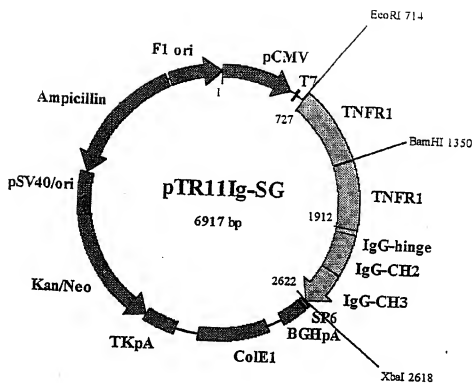
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FIG. 6



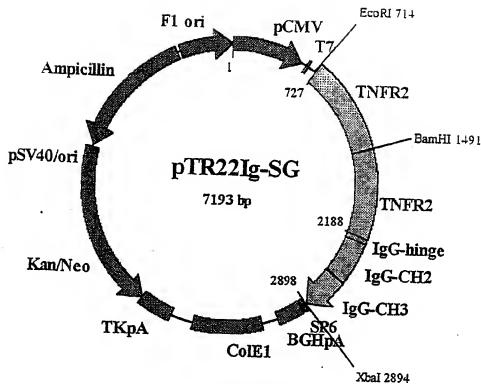
7/23

FIG. 7



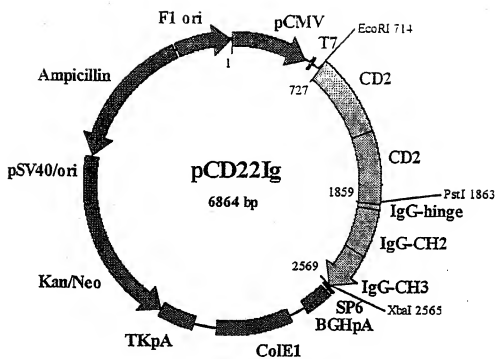
8/23

FIG. 8



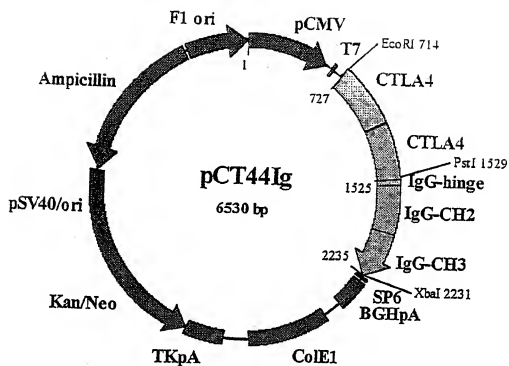
9/23

FIG. 9



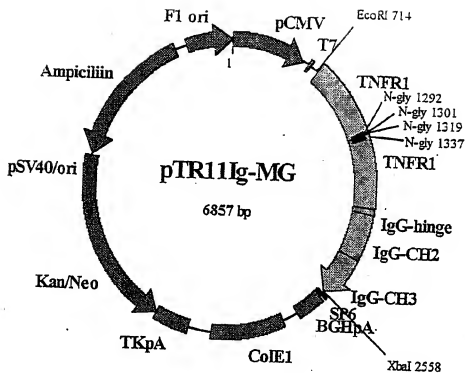
10/23

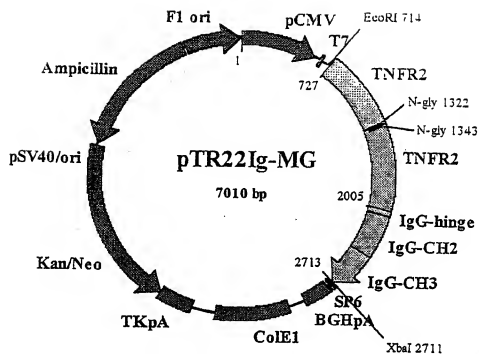
FIG. 10

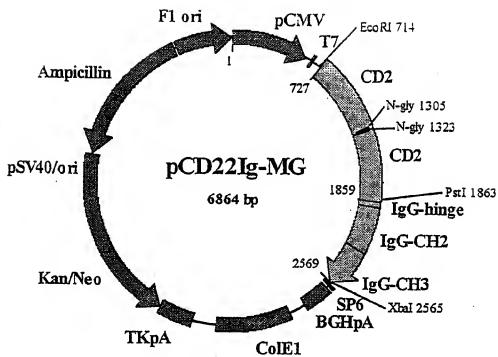


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FIG. 11

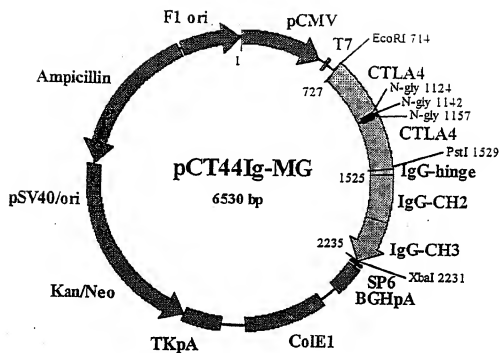


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FIG. 12

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FIG. 13

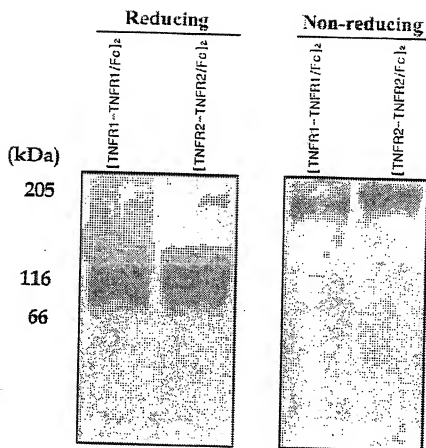
14/23

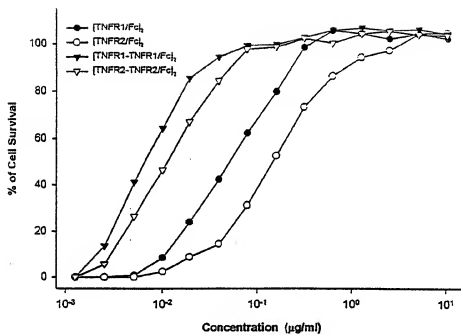
FIG. 14

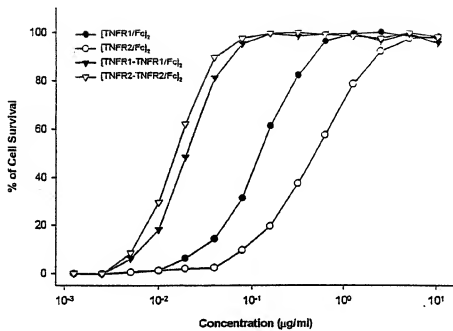


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FIG. 15

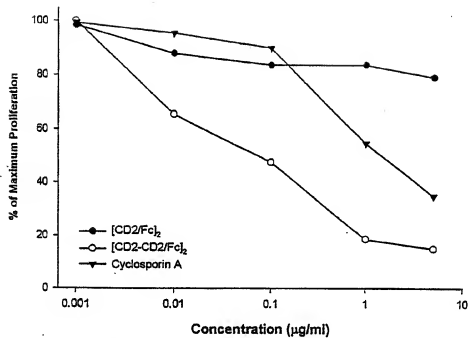


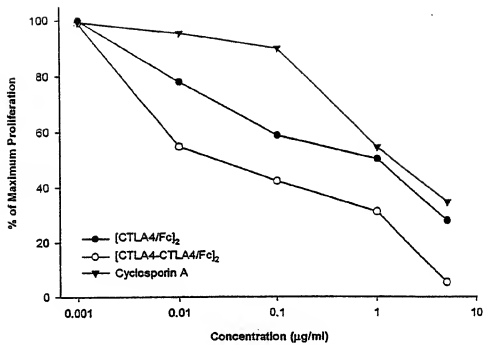
16/23
FIG. 16

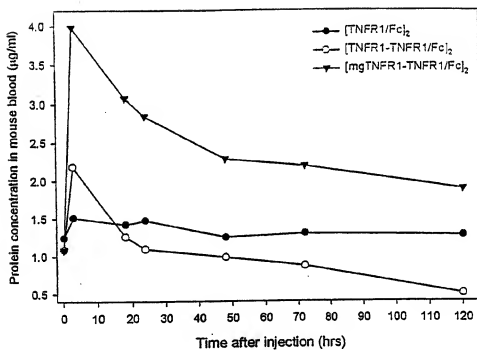
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FIG. 17

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FIG. 18

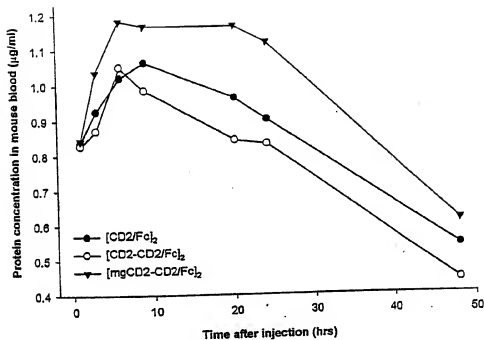


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FIG. 19

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FIG. 20

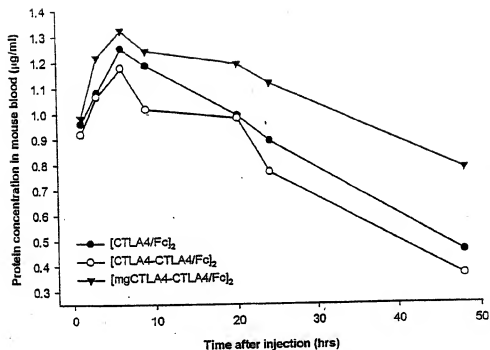
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FIG. 21



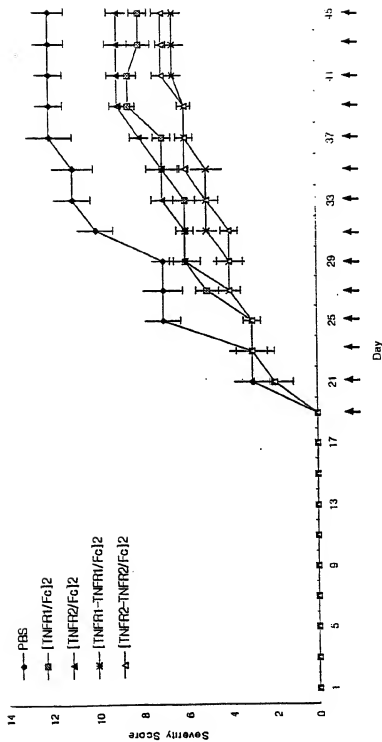
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FIG. 22



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FIG. 23



Sequence Listing

<110> MeDexGen Inc.
CHUNG, Yong Hoon
HAN, Ji Woong
LEE, Hye Ja
CHOI, Eun Yong
KIM, Jin Mi
YIM, Soo Bin

<120> Method of manufacturing Ig-fusion proteins by concatamerization,
TNFR/Fc, CD2/Fc, CTLA4/Fc fusion proteins manufactured by the
method, DNA coding the proteins, vectors including the DNA, and
cells transformed by the vector

<160> 52

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Sequence Listing

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Sequence Listing

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Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
      20          25          30

cac cta ggg gac agg gag aag aga gat agt gtc tgt ccc caa gga aaa      144
His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
      35          40          45

tat atc cac cct caa aat aat tcg att tgc tgt acc aag tgc cac aaa      192
Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
      50          55          60

gga acc tac ttg tac aat gac tgt cca ggc cgg ggg cag gat acg gac      240
Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
      65          70          75          80

tgc agg gag tgt gag agc ggc tcc ttc acc gct tca gaa aac cac ctc      288
Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
      85          90          95

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Sequence Listing

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Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val			
	245	250	255
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Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro			
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Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr			
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Sequence Listing

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Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala		
			325					330					335				
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Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg		
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Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro		
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Ser	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln		
			405					410						415			
ggg	aac	gtc	ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	ctg	cac	aac	cac		1296
Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His		
		420						425				430					
tac	acg	cag	sag	agc	ctc	tcc	ctg	tct	ccg	ggt	aaa			tga			1335
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys						
		435				440											

<210> 2

<211> 444

<212> PRT

<213> Homo sapiens

Sequence Listing

<400> 2

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu

1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro

20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys

35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys

50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp

65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu

85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val

100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg

115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe

130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu

145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu

165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr

180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser

195 200 205

Sequence Listing

```

Gly Thr Thr Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
210                               215                               220

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
225                               230                               235                               240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
                               245                               250                               255

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
                               260                               265                               270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275                               280                               285

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
290                               295                               300

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305                               310                               315                               320

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
                               325                               330                               335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
                               340                               345                               350

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355                               360                               365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
370                               375                               380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
385                               390                               395                               400

Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
                               405                               410                               415

```


Sequence Listing

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 420 425 430

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440

<210> 3
 <211> 1473
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(1470)
 <223> TNFR2-IgG

<220>
 <221> C_region
 <222> (772)..(1473)
 <223> Hinge, CH2, CH3 region

<220>
 <221> misc_signal
 <222> (511)..(519)
 <223> N-linked glycosylation site

<220>
 <221> misc_signal
 <222> (577)..(585)
 <223> N-linked glycosylation site

<220>
 <221> primer_bind
 <222> (1)..(15)
 <223> PCR primer SEQ ID : 29 binding site

Sequence Listing

```

<220>
<221> primer_bind
<222> (754)..(790)
<223> PCR primer SEQ ID : 30(antisense) binding site

<220>
<221> primer_bind
<222> (754)..(790)
<223> PCR primer SEQ ID : 31 binding site

<220>
<221> primer_bind
<222> (1451)..(1473)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(66)
<223> signal peptide

<400> 3
atg gcg ccc gtc gcc gtc tgg gcc gcg ctg gcc gtc gga ctg gag ctc 48
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
1 5 10 15

tgg gct gcg gcg cac gcc tgg ccc gcc cag gtg gca ttt aca ccc tac 96
Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
20 25 30

gcc cgg gag ccc ggg agc aca tgc cgg ctc aga gaa tac tat gac cag 144
Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
35 40 45

aca gct cag atg tgc tgc agc aca tgc tgc cgg ggc caa cat gca aaa 192

```

Sequence Listing

Thr Ala Gln Met Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys			
50	55	60	
gtc ttc tgt acc aag acc tcg gac acc gtg tgt gac tcc tgt gag gac			240
Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp			
65	70	75	80
agc aca tac acc cag ctg tgg aac tgg gtt ccg gag tgc ttg agc tgt			288
Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys			
85	90	95	
ggc tcc cgc tgt agc tct gac cag gtg gaa act caa gcc tgc act cgg			336
Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg			
100	105	110	
gaa cag aac cgc atc tgc acc tgc agg ccc ggc tgg tac tgc gcg ctg			384
Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu			
115	120	125	
agc aag cag gag ggg tgc cgg ctg tgc gcg ccg ctg cgc aag tgc cgc			432
Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg			
130	135	140	
cgg ggc ttc ggc gtg gcc aga cca gga act gaa aca tca gac gtg gtg			480
Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val			
145	150	155	160
tgc aag ccc tgt gcc ccg ggg acg ttc tcc aac acg act tca tcc acg			528
Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Thr Thr			
165	170	175	
gat att tgc agg ccc cac cag atc tgt aac gtg gtg gcc atc cct ggg			576
Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly			
180	185	190	
aat gca agc atg gat gca gtc tgc acg tcc acg tcc ccc acc cgg agt			624
Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser			
195	200	205	
atg gcc cca ggg gca qta cac tta ccc caq cca gtg tcc aca cga tcc			672

Sequence Listing

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser	
210	220
cac cac acg cag cca act cca gaa ccc agc act gct cca agc acc tcc	720
Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser	
225	240
ttc ctg ctc cca atg ggc ccc agc ccc cca gct gaa ggg agc act ggc	768
Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly	
245	255
gac gca gag ccc aaa tot tgt gac aaa act cac aca tgc cca ccg tgc	816
Asp Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys	
260	270
cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca	864
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro	
275	285
aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc	912
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys	
290	300
gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg	960
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp	
305	320
tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag	1008
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu	
325	335
gag cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc ctg	1056
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu	
340	350
cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac	1104
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn	
355	365
aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg	1152

Sequence Listing

```

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
370                375                380

cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag      1200
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
385                390                395                400

ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat      1248
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
405                410                415

ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac      1296
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
420                425                430

aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc tcc ttc      1344
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Ser Phe
435                440                445

ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac      1392
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
450                455                460

gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg      1440
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
465                470                475                480

cag aag agc ctc tcc ctg tct ccg ggt aaa      tga      1473
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485                490

<210> 4
<211> 490
<212> PRT
<213> Homo sapiens

<400> 4
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
1                5                10                15

```

Sequence Listing

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
 20 25 30
 Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
 35 40 45
 Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
 50 55 60
 Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
 65 70 75 80
 Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
 85 90 95
 Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
 100 105 110
 Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
 115 120 125
 Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
 130 135 140
 Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
 145 150 155 160
 Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
 165 170 175
 Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
 180 185 190
 Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
 195 200 205
 Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
 210 215 220

Sequence Listing

```

Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225                      230                      235                      240

Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly
                245                      250                      255

Asp Ala Glu Pro Lys Ser-Cys Asp Lys Thr His Thr Cys Pro Pro Cys
                260                      265                      270

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
                275                      280                      285

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
                290                      295                      300

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
295                      310                      315                      320

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                325                      330                      335

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
                340                      345                      350

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
                355                      360                      365

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                370                      375                      380

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
295                      390                      395                      400

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                405                      410                      415

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
                420                      425                      430

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Ser Phe

```

Sequence Listing

```

435          440          445
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
450          455          460
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
465          470          475          480
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485          490

```

```

<210>      5
<211>      1887
<212>      DNA
<213>      Homo sapiens

<220>
<221>      CDS
<222>      (1)..(1884)
<223>      TNFR1-TNFR1-IgG

<220>
<221>      C_region
<222>      (1716)..(1887)
<223>      Hinge, CH2, CH3 region

<220>
<221>      misc_signal
<222>      (160)..(168)
<223>      N-linked glycosylation site

<220>
<221>      misc_signal
<222>      (433)..(441)
<223>      N-linked glycosylation site

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Sequence Listing

<220>
<221> misc_signal
<222> (451)..(459)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (631)..(639)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (712)..(720)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (985)..(993)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (1003)..(1011)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 25 binding site

<220>
<221> primer_bind
<222> (592)..(628)

Sequence Listing

```

<223>    PCR primer SEQ ID : 33(antisense) binding site

<220>
<221>    primer_bind
<222>    (622)..(655)
<223>    PCR primer SEQ ID : 32 binding site

<220>
<221>    primer_bind
<222>    (1168)..(1204)
<223>    PCR primer SEQ ID : 26(antisense) binding site

<220>
<221>    primer_bind
<222>    (1168)..(1204)
<223>    PCR primer SEQ ID : 27 binding site

<220>
<221>    primer_bind
<222>    (1864)..(1887)
<223>    PCR primer SEQ ID : 28(antisense) binding site

<220>
<221>    sig_peptide
<222>    (1)..(60)
<223>    signal peptide

<400>      5
atg ggc ctc tcc acc gtg cct gac ctg ctg ctg cgg ctg gtg ctc ctg      48
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
      1          5          10          15

gag ctg ttg gtg gga ata tac ccc tca ggg gtt att gga ctg gtc cct      96
Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro

```

Sequence Listing

20	25	30	
cac cta ggg gac agg gag aag aga gat agt gtg tgt ccc caa gga aaa			144
His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys			
35	40	45	
tat atc cac cct caa aat aat tcg att tgc tgt acc aag tgc cac aaa			192
Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys			
50	55	60	
gga acc tac ttg tac aat gac tgt cca ggc cgg ggg cag gat acg gac			240
Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp			
65	70	75	80
tgc agg gag tgt gag agc ggc tcc ttc acc gct tca gaa aac cac ctc			288
Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu			
85	90	95	
aga cac tgc ctc agc tgc tcc aaa tgc cga aag gaa atg ggt cag gtg			336
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val			
100	105	110	
gag atc tct tct tgc aca gtg gac cgg gac acc gtg tgt ggc tgc agg			384
Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg			
115	120	125	
aag aac cag tac cgg cat tat tgg agt gaa aac ctt ttc cag tgc ttc			432
Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe			
130	135	140	
aat tgc agc ctc tgc ctc aat ggg acc gtg cac ctc tcc tgc cag gag			480
Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu			
145	150	155	160
aaa cag aac acc gtg tgc acc tgc cat gca ggt ttc ttt cta aga gaa			528
Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu			
165	170	175	
aac gag tgt gtc tcc tgt agt aac tgt aag aaa agc ctg gag tgc acg			576
Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr			

Sequence Listing

180	185	190	
aag ttg tgc cta ccc cag att gag aat gtt aag ggc act gag gac gga			624
Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Gly			
195	200	205	
tcc ggg aac att tca ctg gtc cct cac cta ggg gac agg gag aag aga			672
Ser Gly Asn Ile Ser Leu Val Pro His Leu Gly Asp Arg Glu Lys Arg			
210	215	220	
gat agt gtg tgt ccc caa gga aaa tat atc cac cct caa aat aat tcg			720
Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser			
225	230	235	240
att tgc tgt acc aag tgc cac aaa gga acc tac ttg tac aat gac tgt			768
Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys			
245	250	255	
cca ggc ccg ggg cag gat acg gac tgc agg gag tgt gag agc ggc tcc			816
Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser			
260	265	270	
ttc acc gct tca gaa aac cac ctc aga cac tgc ctc agc tgc tcc aaa			864
Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys			
275	280	285	
tgc cga aag gaa atg ggt cag gtg gag atc tct tct tgc aca gtg gac			912
Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp			
290	295	300	
cgg gac acc gtg tgt ggc tgc agg aag aac cag tac cgg cat tat tgg			960
Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp			
305	310	315	320
agt gaa aac ctt ttc cag tgc ttc aat tgc agc ctc tgc ctc aat ggg			1008
Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly			
325	330	335	
acc gtg cac ctc tcc tgc cag gag aaa cag aac acc gtg tgc acc tgc			1056
Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys			

Sequence Listing

340	345	350	
cat gca ggt ttc ttt cta aga gaa aac gag tgt gtc tcc tgt agt aac			1104
His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn			
355	360	365	
tgt aag aaa agc ctg gag tgc acg aag ttg tgc cta ccc cag att gag			1152
Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu			
370	375	380	
aat gtt aag ggc act gag gac tca ggc acc aca gca gag ccc aaa tct			1200
Asn Val Lys Gly Thr Glu Asp Ser Gly Thr Thr Ala Glu Pro Lys Ser			
385	390	395	400
tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg			1240
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu			
405	410	415	
ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc			1296
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu			
420	425	430	
atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc			1344
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser			
435	440	445	
cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag			1392
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu			
450	455	460	
gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg			1440
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr			
465	470	475	480
tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat			1480
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn			
485	490	495	
ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc			1536
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro			

Sequence Listing

500	505	510	
atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag			1584
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln			
515	520	525	
gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc			1632
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val			
530	535	540	
agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg			1680
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val			
545	550	555	560
gag tgg gag agc aat ggg cag cgg gag aac aac tac aag acc acg cct			1728
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro			
565	570	575	
ccc gtg ctg gac tcc gac ggc tcc tcc ttc ctc tac agc aag ctc acc			1776
Pro Val Leu Asp Ser Asp Gly Ser Ser Phe Leu Tyr Ser Lys Leu Thr			
580	585	590	
gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg			1824
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val			
595	600	605	
atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg			1872
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu			
610	615	620	
tct ccg ggt aaa	tga		1887
Ser Pro Gly Lys			
625			
<210> 6			
<211> 628			
<212> PRT			
<213> Homo sapiens			

Sequence Listing

<400> 6

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu

1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro

20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys

35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys

50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp

65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu

85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val

100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg

115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe

130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu

145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu

165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr

180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Gly

195 200 205

Sequence Listing

```

Ser Gly Asn Ile Ser Leu Val Pro His Leu Gly Asp Arg Glu Lys Arg
  210                215                220

Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
  225                230                235                240

Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys
      245                250                255

Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser
      260                265                270

Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys
      275                280                285

Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp
      290                295                300

Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp
      305                310                315                320

Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly
      325                330                335

Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys
      340                345                350

His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn
      355                360                365

Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu
      370                375                380

Asn Val Lys Gly Thr Glu Asp Ser Gly Thr Thr Ala Glu Pro Lys Ser
      385                390                395                400

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
      405                410                415

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu

```


Sequence Listing

420	425	430
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
435	440	445
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
450	455	460
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
465	470	475
		480
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
485	490	495
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
500	505	510
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
515	520	525
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val		
530	535	540
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
545	550	555
		560
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
565	570	575
Pro Val Leu Asp Ser Asp Gly Ser Ser Phe Leu Tyr Ser Lys Leu Thr		
580	585	590
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
595	600	605
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
610	615	620
Ser Pro Gly Lys		
625		

Sequence Listing

<210> 7
<211> 2163
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(2160)
<223> TNFR2-TNFR2-IgG

<220>
<221> C_region
<222> (1462)..(2163)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (511)..(519)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (577)..(585)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (769)..(777)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (1201)..(1209)

Sequence Listing

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (1267)..(1275)

<223> N-linked glycosylation site

<220>

<221> primer_bind

<222> (1)..(15)

<223> PCR primer SEQ ID : 29 binding site

<220>

<221> primer_bind

<222> (761)..(795)

<223> PCR primer SEQ ID : 35(antisense) binding site

<220>

<221> primer_bind

<222> (741)..(766)

<223> PCR primer SEQ ID : 34 binding site

<220>

<221> primer_bind

<222> (1444)..(1480)

<223> PCR primer SEQ ID : 30(antisense) binding site

<220>

<221> primer_bind

<222> (1444)..(1480)

<223> PCR primer SEQ ID : 31 binding site

<220>

Sequence Listing

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<221> primer_bind
<222> (2141)..(2163)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(66)
<223> signal peptide

<400> 7
atg gcg ccc gtc gcc gtc tgg gcc gcg ctg gcc gtc gga ctg gag ctc 48
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
1 5 10 15

tgg gct gcg gcg cac gcc ttg ccc gcc cag gtg gca ttt aca ccc tac 96
Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
20 25 30

gcc ccg gag ccc ggg agc aca tgc cgg ctc aga gaa tac tat gac cag 144
Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
35 40 45

aca gct cag atg tgc tgc agc aaa tgc tgc ccg gcc caa cat gca aaa 192
Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
50 55 60

gtc ttc tgt acc aag acc tcg gac acc gtg tgt gac tcc tgt gag gac 240
Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65 70 75 80

agc aca tac acc cag ctc tgg aac tgg gtt ccc gag tgc ttg agc tgt 288
Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
85 90 95

ggc tcc cgc tgt agc tct gac cag gtg gaa act caa gcc tgc act ccg 336
Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
100 105 110

```

Sequence Listing

gaa cag aac cgc atc tgc acc tgc agg ccc ggc tgg tac tgc gcg ctg Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu 115 120 125	384
agc aag cag gag ggg tgc cgg ctg tgc gcg cgg ctg cgc aag tgc cgc Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg 130 135 140	432
ccg ggc ttc ggc gtg gcc aga cca gga act gaa aca tca gac gtg gtg Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val 145 150 155 160	480
tgc aag ccc tgt gcc cgg ggg acg ttc tcc aac acg act tca tcc acg Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr 165 170 175	528
gat att tgc agg ccc cac cag atc tgt aac gtg gtg gcc atc cct ggg Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly 180 185 190	576
aat gca agc atg gat gca gtc tgc acg tcc acg tcc ccc acc cgg agt Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser 195 200 205	624
atg gcc cca ggg gca gta cac tta ccc cag cca gtg tcc aca cga tcc Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser 210 215 220	672
caa cac acg cag cca act cca gaa ccc agc act gct cca agc acc tcc Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser 225 230 235 240	720
ttc ctg ctg cca atg ggc ccc agc ccc cca gct gaa ggg agc gga tcc Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Gly Ser 245 250 255	768
aac gca act aca ccc tac gcc cgg gag ccc ggg agc aca tgc cgg ctg Asn Ala Thr Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu 260 265 270	816

Sequence Listing

aga gaa tac tat gac cag aca gct cag atg atg tgc agc aac tgc tgc	864
Arg Glu Tyr Tyr Asp Glu Thr Ala Gln Met Cys Cys Ser Lys Cys Ser	
275 280 285	
ccg ggc caa cat gca aaa gtc ttc tgt acc aag acc tgc gac acc gtg	912
Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser Asp Thr Val	
290 295 300	
tgt gac tcc tgt gag gac agc aca tac acc cag ctc tgg aac tgg gtt	960
Cys Asp Ser Cys Glu Ser Cys Gly Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val	
305 310 315 320	
ccc gag tgc ttg agc tgt ggc tcc cgc tgt agc tct gac cag gtg gaa	1008
Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp Gln Val Glu	
325 330 335	
act caa gcc tgc act cgg gaa cag aac cgc atc tgc acc tgc agg ccc	1056
Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro	
340 345 350	
ggc tgg tac tgc gcg ctg agc aag cag gag ggg tgc cgg ctg tgc gcg	1104
Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala	
355 360 365	
ccg ctg cgc aag tgc cgc ccg ggc ttc ggc gtg gcc aga cca gga act	1152
Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg Pro Gly Thr	
370 375 380	
gaa aca tca gac gtg gtg tgc aag ccc tgt gcc ccg ggy acg ttc tcc	1200
Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser	
385 390 395 400	
aac acg act tca tcc acg gat att tgc agg ccc cac cag atc tgt aac	1248
Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln Ile Cys Asn	
405 410 415	
gtg gtg gcc atc cct ggg aat gca agc atg gat gca gtc tgc aag tcc	1296
Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val Cys Thr Ser	
420 425 430	

Sequence Listing

acg tcc ccc acc cgg agt atg gcc cca ggg gca gta cac tta ccc cag	1344
Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His Leu Pro Gln	
435 440 445	
cca gtg tcc aca cga tcc cca cac acg cag cca act cca gaa ccc agc	1392
Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro Glu Pro Ser	
450 455 460	
act gct cca agc acc tcc ttc ctg ctc cca atg ggc ccc agc ccc cca	1440
Thr Ala Pro Ser Thr Thr Ser Phe Leu Leu Pro Met Gly Pro Ser Pro Pro	
465 470 475 480	
gct gaa ggg agc act ggc gac gca gag ccc aaa tct tgt gac aaa act	1488
Ala Glu Gly Ser Thr Gly Asp Ala Glu Pro Lys Ser Cys Asp Lys Thr	
485 490 495	
cac aca tgc cca cgg tgc cca gca cct gaa ctc ctg ggg gga cgg tca	1536
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser	
500 505 510	
gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg	1584
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg	
515 520 525	
acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct	1632
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro	
530 535 540	
gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc	1680
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala	
545 550 555 560	
aag aca aag cgg cgg gag gag cag tac aac agc acg tac cgg gtg gtc	1728
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val	
565 570 575	
agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac	1776
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr	
580 585 590	

Sequence Listing

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aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc      1824
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
595                      600                      605

atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg      1872
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
610                      615                      620

ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc      1920
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
625                      630                      635                      640

ctg gtc aaa gcc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc      1968
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
645                      650                      655

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac      2016
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
660                      665                      670

tcc gac gcc tcc tcc ttc ctc tac agc aag ctc acc gtg gac aag agc      2064
Ser Asp Gly Ser Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
675                      680                      685

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct      2112
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
690                      695                      700

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa      2160
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
705                      710                      715                      720

tga                                                                    2163

```

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<210> 8
<211> 720
<212> PRT
<213> Homo sapiens

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Sequence Listing

<400> 8

Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu

1 5 10 15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr

20 25 30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln

35 40 45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys

50 55 60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp

65 70 75 80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys

85 90 95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg

100 105 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu

115 120 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg

130 135 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val

145 150 155 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr

165 170 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly

180 185 190

Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser

195 200 205

Sequence Listing

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
 210 215 220

Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
 225 230 235 240

Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Gly Ser
 245 250 255

Asn Ala Thr Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu
 260 265 270

Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys Ser Lys Cys Ser
 275 280 285

Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser Asp Thr Val
 290 295 300

Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val
 305 310 315 320

Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp Gln Val Glu
 325 330 335

Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro
 340 345 350

Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala
 355 360 365

Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg Pro Gly Thr
 370 375 380

Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser
 385 390 395 400

Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln Ile Cys Asn
 405 410 415

Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val Cys Thr Ser

Sequence Listing

420	425	430
Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His Leu Pro Gln		
435	440	445
Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro Glu Pro Ser		
450	455	460
Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly Pro Ser Pro Pro		
465	470	475
480		
Ala Glu Gly Ser Thr Gly Asp Ala Glu Pro Lys Ser Cys Asp Lys Thr		
485	490	495
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser		
500	505	510
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg		
515	520	525
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro		
530	535	540
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala		
545	550	555
560		
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val		
565	570	575
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr		
580	585	590
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr		
595	600	605
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu		
610	615	620
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys		
625	630	635
640		

Sequence Listing

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
645 650 655

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
660 665 670

Ser Asp Gly Ser Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
675 680 685

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
690 695 700

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
705 710 715 720

<210> 9
<211> 1827
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1824)
<223> mgTNFR1-TNFR1-IgG

<220>
<221> C_region
<222> (1126)..(1827)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (160)..(168)
<223> N-linked glycosylation site

Sequence Listing

<220>
<221> misc_signal
<222> (433)..(441)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (451)..(459)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (565)..(573)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (574)..(582)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (592)..(600)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (610)..(618)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (925)..(933)
<223> N-linked glycosylation site

Sequence Listing

<220>
<221> misc_signal
<222> (943)..(951)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 25 binding site

<220>
<221> primer_bind
<222> (545)..(606)
<223> PCR primer SEQ ID : 37(antisense) binding site

<220>
<221> primer_bind
<222> (559)..(621)
<223> PCR primer SEQ ID : 36 binding site

<220>
<221> primer_bind
<222> (1108)..(1144)
<223> PCR primer SEQ ID : 26(antisense) binding site

<220>
<221> primer_bind
<222> (1108)..(1144)
<223> PCR primer SEQ ID : 27 binding site

<220>
<221> primer_bind

Sequence Listing

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<222>      (1804)..(1827)
<223>      PCR primer SEQ ID : 28(antisense) binding site

<220>
<221>      sig_peptide
<222>      (1)..(60)
<223>      signal peptide

<400>      9
atg ggc ctc tcc acc gtg cct gac ctg ctg ctg ccg ctg gtg ctc ctg      48
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
      1          5          10          15

gag ctg ttg gtg gga ata tac ccc tca ggg gtt att gga ctg gtc cct      96
Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
      20          25          30

cac cta ggg gac agg gag aag aga gat agt gtg tgt ccc caa gga aaa      144
His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
      35          40          45

tat atc cac cct caa aat aat tgg att tgc tgt acc aag tgc cac aaa      192
Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
      50          55          60

gga acc tac ttg tac aat gac tgt cca ggc ccg ggg cag gat acg gac      240
Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
      65          70          75          80

tgc agg gag tgt gag ayc ggc tcc ttc acc gct tca gaa aac cac etc      288
Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
      85          90          95

aga cac tgc ctc agc tgc tcc aaa tgc cga aag gaa atg ggt cag gtg      336
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
      100          105          110

gag atc tct tct tgc aca gtg gac cgg gac acc gtg tgt ggc tgc agg      384

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Sequence Listing

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg	
115	125
aag aac cag tac cgg cat tat tgg agt gaa aac ctt ttc cag tgc ttc	432
Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe	
130	140
aat tgc agc ctc tgc ctc aat ggg acc gtg cac ctc tcc tgc cag gag	480
Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu	
145	155
aaa cag aac acc gtg tgc acc tgc cat gca ggt ttc ttt cta aga gaa	528
Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu	
165	175
aac gag tgt gtc tcc tgt agt aac tgt aag aaa agc aac gag acc aac	576
Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Asn Glu Thr Asn	
180	195
aag acc tgc cta cac aac ggg tcc agg gag aag aac gat agt gtg tgt	624
Lys Thr Cys Leu His Asn Gly Ser Arg Glu Lys Asn Asp Ser Val Cys	
195	205
ccc caa gga aaa tat atc cac cct caa aat aat tgc att tgc tgt acc	672
Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr	
210	220
aag tgc cac aaa gga acc tac ttg tac aat gac tgt cca ggc cgg ggg	720
Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly	
225	235
cag gat acg gac tgc agg gag tgt gag agc ggc tcc ttc acc gct tca	768
Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Phe Thr Ala Ser	
245	255
gaa aac cac ctc aga cac tgc ctc agc tgc tcc aaa tgc cga aag gaa	816
Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu	
260	270
atg ggt cag gtg gag atc tct tct tgc aca gtg gac cgg gac acc gtg	864

Sequence Listing

Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val	
275	280 285
tgt ggc tgc agg aag aac cag tac cgg cat tat tgg agt gaa aac ctt	912
Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu	
290	295 300
ttc cag tgc ttc aat tgc agc ctc tgc ctc aat ggg acc gtg cac ctc	960
Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu	
305	310 315 320
tcg tgc cag gag aaa cag aac acc gtg tgc acc tgc cat gca ggt ttc	1008
Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe	
325	330 335
ttt cta aga gaa aac gag tgt gtc tcc tgt agt aac tgt aag aaa agc	1056
Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser	
340	345 350
ctg gag tgc acg aag ttg tgc cta ccc cag att gag aat gtt aag ggc	1104
Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly	
355	360 365
act gag gac tca ggc acc aca gca gag ccc aaa tct tgt gac aaa act	1152
Thr Glu Asp Ser Gly Thr Thr Ala Glu Pro Lys Ser Cys Asp Lys Thr	
370	375 380
cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca	1200
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser	
385	390 395 400
gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg	1248
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg	
405	410 415
acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct	1296
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro	
420	425 430
gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aal gcc	1344

Sequence Listing

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala	
435 440 445	
aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg gtc	1392
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val	
450 455 460	
agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac	1440
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr	
465 470 475 480	
aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc	1488
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr	
485 490 495	
atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg	1536
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu	
500 505 510	
ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc	1584
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys	
515 520 525	
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	1632
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser	
530 535 540	
aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1680
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp	
545 550 555 560	
tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc	1728
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser	
565 570 575	
agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1776
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala	
580 585 590	
ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa	1824

Sequence Listing

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

595

600

605

tga

1827

<210> 10

<211> 608

<212> PRT

<213> Homo sapiens

<400> 10

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu

1

5

10

15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro

20

25

30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys

35

40

45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys

50

55

60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp

65

70

75

80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu

85

90

95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val

100

105

110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg

115

120

125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe

130

135

140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu

Sequence Listing

145	150	155	160
Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu			
165	170	175	
Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Asn Glu Thr Asn			
180	185	190	
Lys Thr Cys Leu His Asn Gly Ser Arg Glu Lys Asn Asp Ser Val Cys			
195	200	205	
Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr			
210	215	220	
Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly			
225	230	235	240
Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser			
245	250	255	
Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu			
260	265	270	
Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val			
275	280	285	
Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu			
290	295	300	
Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu			
305	310	315	320
Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe			
325	330	335	
Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser			
340	345	350	
Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly			
355	360	365	

Sequence Listing

Thr Glu Asp Ser Gly Thr Thr Ala Glu Pro Lys Ser Cys Asp Lys Thr
 370 375 380

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
 385 390 395 400

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
 405 410 415

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
 420 425 430

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
 435 440 445

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
 450 455 460

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
 465 470 475 480

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
 485 490 495

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
 500 505 510

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
 515 520 525

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
 530 535 540

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
 545 550 555 560

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
 565 570 575

Sequence Listing

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
 580 585 590

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 595 600 605

<210> 11
 <211> 1980
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(1977)
 <223> mgTNFR2-TNFR2-IgG

<220>
 <221> C_region
 <222> (1279)..(1980)
 <223> Hinge, CH2, CH3 region

<220>
 <221> misc_signal
 <222> (511)..(519)
 <223> N-linked glycosylation site

<220>
 <221> misc_signal
 <222> (577)..(585)
 <223> N-linked glycosylation site

<220>
 <221> misc_signal

Sequence Listing

<222> (595)..(603)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (616)..(624)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (1018)..(1026)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (1084)..(1092)

<223> N-linked glycosylation site

<220>

<221> primer_bind

<222> (1)..(15)

<223> PCR primer SEQ ID : 29 binding site

<220>

<221> primer_bind

<222> (586)..(627)

<223> PCR primer SEQ ID : 39(antisense) binding site

<220>

<221> primer_bind

<222> (586)..(630)

<223> PCR primer SEQ ID : 38 binding site

Sequence Listing

<220>
 <221> primer_bind
 <222> (1261)..(1296)
 <223> PCR primer SEQ ID : 30(antisense) binding site

<220>
 <221> primer_bind
 <222> (1261)..(1296)
 <223> PCR primer SEQ ID : 31 binding site

<220>
 <221> primer_bind
 <222> (1957)..(1980)
 <223> PCR primer SEQ ID : 28(antisense) binding site

<220>
 <221> sig_peptide
 <222> (1)..(66)
 <223> signal peptide

<400> 11
 atg gcg ccc gtc gcc gtc tgg gcc gcg ctg gcc gtc gga ctg gag ctc 48
 Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
 1 5 10 15
 tgg gct gcg gcg cac gcc ttg ccc gcc cag gtg gca ttt aca ccc tac 96
 Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
 20 25 30
 gcc ccg gag ccc ggg agc aca tgc cgg ctc aga gaa tac tat gac cag 144
 Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Asp Gln
 35 40 45
 aca gct cag atg tgc tgc agc aaa tgc tcg ccg gcc caa cat gca aaa 192
 Thr Ala Gln Met Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
 50 55 60

Sequence Listing

gtc ttc tgt acc aag acc tcg gac acc gtg tgt gac tcc tgt gag gac Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp 65 70 75 80	240
agc aca tac acc cag ctc tgg aac tgg gtt ccc gag tgc ttg agc tgt Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys 85 90 95	288
ggc tcc cgc tgt agc tct gac cag gtg gaa act caa gcc tgc act cgg Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg 100 105 110	336
gaa cag aac cgc atc tgc acc tgc agg ccc ggc tgg tac tgc gcg ctg Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu 115 120 125	384
agc aag cag gag ggg tgc cgg ctg tgc gcg cgg ctg cgc aag tgc cgc Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg 130 135 140	432
ccg ggc ttc ggc gtg gcc aga cca gga act gaa aca tca gac gtg gtg Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val 145 150 155 160	480
tgc aag ccc tgt gcc cgg ggg acg ttc tcc aac acg act tca tcc acg Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr 165 170 175	528
gat att tgc agg ccc cac cag atc tgt aac gtg gtg gcc atc cct ggg Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly 180 185 190	576
aat gca agc atg gat gca aac tgc acg tcc cgg gag ccc aac agc aca Asn Ala Ser Met Asp Ala Asn Cys Thr Ser Pro Glu Pro Asn Ser Thr 195 200 205	624
tgc cgg ctc aga gaa tac tat gac cag aca gct cag atg tgc tgc agc Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys Ser 210 215 220	672

Sequence Listing

aaa tgc tgc ccg ggc caa cat gca aaa gtc ttc tgt acc aag acc tgc Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser 225 230 235 240	720
gac acc gtg tgt gac tcc tgt gag gac agc aca tac acc cag ctc tgg Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu Trp 245 250 255	760
aac tgg gtt ccc gag tgc ttg agc tgt ggc tcc cgc tgt agc tct gac Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp 260 265 270	816
cag gtg gaa act caa gcc tgc act cgg gaa cag aac cgc atc tgc acc Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr 275 280 285	864
tgc agg ccc cgc tgg tac tgc gcg ctg agc aag cag gag ggg tgc cgg Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg 290 295 300	912
ctg tgc gcg ccg ctg cgc aag tgc cgc ccg ggc ttc gcc gtg gcc aga Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg 305 310 315 320	960
cca gga act gaa aca tca gac gtg gtg tgc aag ccc tgt gcc ccg ggg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly 325 330 335	1008
acg ttc tcc aac acg act tca tcc acg gat att tgc agg ccc cac cag Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln 340 345 350	1056
atc tgt aac gtg gtg gcc atc cct ggg aat gca agc atg gat gca gtc Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val 355 360 365	1104
tgc acg tcc acg tcc ccc acc cgg agt atg gcc cca ggg gca gta cac Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His 370 375 380	1152

Sequence Listing

ttt ccc cag cca gty tcc aca cga tcc caa cac acg cag cca act cca Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro 385 390 395 400	1200
gaa ccc agc act gct cca agc acc tcc ttc ctg ctc cca atg ggc ccc Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly Pro 405 410 415	1248
agc ccc cca gct gaa ggg agc act ggc gac gca gag ccc aaa tct tgt Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Ala Glu Pro Lys Ser Cys 420 425 430	1296
gac aaa act cac aca tgc cca cgg tgc cca gca cct gaa ctc ctg ggg Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly 435 440 445	1344
gga cgg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met 450 455 460	1392
atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His 465 470 475 480	1440
gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val 485 490 495	1488
cat aat gcc aag aca aag cgg cgg gag gag cag tac aac agc acg tac His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr 500 505 510	1536
cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat gcc Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly 515 520 525	1584
aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile 530 535 540	1632

Sequence Listing

```

gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg      1680
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
545                550                555                560

tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc      1728
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
                565                570                575

ctg acc tgc ctg gtc aaa gcc ttc tat ccc agc gac atc gcc gtg gag      1776
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
                580                585                590

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc      1824
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                595                600                605

gtg ctg gac tcc gac gcc tcc ttc ttc ctg tac agc aag ctg acc gtg      1872
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
        610                615                620

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg      1920
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        625                630                635                640

cat gag gct ctg cac aac cac tac acg cag aag agc ctg tcc ctg tct      1968
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
                645                650                655

ccg ggt aaa          tga          1980
Pro Gly Lys

```

<210> 12

<211> 659

<212> PRT

<213> Homo sapiens

<400> 12

Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu

Sequence Listing

```

1           5           10          15
Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
           20           25           30
Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
           35           40           45
Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
           50           55           60
Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
           65           70           75           80
Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
           85           90           95
Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
          100          105          110
Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
          115          120          125
Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
          130          135          140
Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
          145          150          155          160
Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
          165          170          175
Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
          180          185          190
Asn Ala Ser Met Asp Ala Asn Cys Thr Ser Pro Glu Pro Asn Ser Thr
          195          200          205
Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys Ser
          210          215          220

```

Sequence Listing

Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser
225 230 235 240

Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu Trp
245 250 255

Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp
260 265 270

Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr
275 280 285

Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg
290 295 300

Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg
305 310 315 320

Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly
325 330 335

Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln
340 345 350

Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val
355 360 365

Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His
370 375 380

Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro
385 390 395 400

Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly Pro
405 410 415

Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Ala Glu Pro Lys Ser Cys
420 425 430

Sequence Listing

```

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
    435                      440                      445

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
    450                      455                      460

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
    465                      470                      475                      480

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    485                      490                      495

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
    500                      505                      510

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
    515                      520                      525

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
    530                      535                      540

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
    545                      550                      555                      560

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    565                      570                      575

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    580                      585                      590

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
    595                      600                      605

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
    610                      615                      620

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
    625                      630                      635                      640

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser

```

Sequence Listing

645

650

655

Pro Gly Lys

<210> 13

<211> 1314

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1311)

<223> CD2-IgG

<220>

<221> C_region

<222> (613)..(1314)

<223> Hinge, CH2, CH3 region

<220>

<221> misc_signal

<222> (265)..(273)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (421)..(429)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (448)..(456)

<223> N-linked glycosylation site

Sequence Listing

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<220>
<221> primer_bind
<222> (1)..(27)
<223> PCR primer SEQ ID : 40 binding site

<220>
<221> primer_bind
<222> (589)..(618)
<223> PCR primer SEQ ID : 41(antisense) binding site

```

```

<220>
<221> primer_bind
<222> (611)..(633)
<223> PCR primer SEQ ID : 42 binding site

```

```

<220>
<221> primer_bind
<222> (1292)..(1314)
<223> PCR primer SEQ ID : 28(antisense) binding site

```

```

<220>
<221> sig_peptide
<222> (1)..(72)
<223> signal peptide

```

```

<400> 13
atg agc ttt cca tgt aaa ttt gta gcc agc ttc ctt ctg att ttc aat      48
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
1          5          10          15

ggt tct tcc aaa ggt gca gtc tcc aaa gag att acg aat gcc ttg gaa      96
Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
20          25          30

```

Sequence Listing

acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac att cct agt ttt	144
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe	
35 40 45	
caa atg sgt gat gat att gac gat ata aaa tgg gaa aaa act tca gac	192
Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp	
50 55 60	
aag aaa aag att gca caa ttc aga aaa gag aaa gag act ttc aag gaa	240
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu	
65 70 75 80	
aaa gat aca tat aag cta ttt aaa aat gga act ctg aaa att aag cat	288
Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His	
85 90 95	
ctg aag acc gat gat cag gat atc tac aag gta tca ata tat gat aca	336
Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr	
100 105 110	
aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg aag att caa gag	384
Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu	
115 120 125	
agg gtc tca aaa cca aag atc tcc tgg act tgt atc aac aca acc ctg	432
Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu	
130 135 140	
acc tgt gag gta atg aat gga act gac ccc gaa tta aac ctg tat caa	480
Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln	
145 150 155 160	
gat ggg aaa cat cta aaa ctt tct cag agg gtc atc aca cac aag tgg	528
Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp	
165 170 175	
acc acc agc ctg agt gca aaa ttc aag tgc aca gca ggg aac aaa gtc	576
Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val	
180 185 190	

Sequence Listing

agc aag gaa tcc agt gtc gag cct gtc agc tgt cct gca gag ccc aaa	624
Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Ala Glu Pro Lys	
195 200 205	
tct tgt gac aaa act cac aca tgc cca cgg tgc cca gca cct gaa ctc	672
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu	
210 215 220	
ctg ggg gga cgg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc	720
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr	
225 230 235 240	
ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg	768
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val	
245 250 255	
agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg	816
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val	
260 265 270	
gag gtg cat aat gcc aag aca aag cgg cgg gag gag cag tac aac agc	864
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser	
275 280 285	
acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg	912
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu	
290 295 300	
aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc	960
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala	
305 310 315 320	
ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca	1008
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro	
325 330 335	
cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag	1056
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln	
340 345 350	

Sequence Listing

```

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc      1104
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
      355              360              365

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg      1152
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
      370              375              380

ccr ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc      1200
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
      385              390              395              400

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc      1248
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
      405              410              415

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc      1296
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
      420              425              430

ctg tct cag ggt aac tga      1314
Leu Ser Pro Gly Lys
      435

<210> 14
<211> 437
<212> PRT
<213> Homo sapiens

<400> 14
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
  1             5             10             15

Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
      20             25             30

Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
      35             40             45

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Sequence Listing

Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
50 55 60

Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
65 70 75 80

Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
85 90 95

Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
100 105 110

Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
115 120 125

Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
130 135 140

Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
145 150 155 160

Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
165 170 175

Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
180 185 190

Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Ala Glu Pro Lys
195 200 205

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
210 215 220

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
225 230 235 240

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
245 250 255

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val

Sequence Listing

```

                260                265                270
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    275                280                285

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
    290                295                300

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
    305                310                315                320

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
    325                330                335

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
    340                345                350

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    355                360                365

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    370                375                380

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
    385                390                395                400

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
    405                410                415

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    420                425                430

Leu Ser Pro Gly Lys
    435

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<210> 15
<211> 1134
<212> DNA
<213> Homo sapiens

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Sequence Listing

<220>
<221> CDS
<222> (1)..(1131)
<223> CTLA4-IgG

<220>
<221> C_region
<222> (433)..(1134)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (289)..(297)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (385)..(393)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 43 binding site

<220>
<221> primer_bind
<222> (409)..(438)
<223> PCR primer SEQ ID : 44(antisense) binding site

<220>
<221> primer_bind
<222> (430)..(453)

Sequence Listing

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<223> PCR primer SEQ ID : 42 binding site

<220>
<221> primer_bind
<222> (1111)..(1134)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(63)
<223> signal peptide

<400> 15
atg agg acc tgg ccc tgc act ctc ctg ttt ttt ctt ctc ttc atc cct 48
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
1 5 10 15

gtc ttc tgc aaa gca atg cac gtg gcc cag cct gct gtg gta ctg gcc 96
Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
20 25 30

agc agc cga ggc atc gcc agc ttt gtg tgt gag tat gca tct cca ggc 144
Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
35 40 45

aaa gcc act gag gtc cgg gtg aca gtg ctt cgg cag gct gac agc cag 192
Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
50 55 60

gtg act gaa gtc tgt gcg gca acc tac atg atg ggg aat gag ttg acc 240
Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
65 70 75 80

ttc cta gat gat tcc atc tgc acg gcc acc tcc agt gga aat caa gtg 288
Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
85 90 95

```


Sequence Listing

aac ctc act atc caa gga ctg agg gcc atg gac acg gga ctc tac atc Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile 100 105 110	336
tgc aag gtg gag ctc atg tac cca cgg cca tac tac ctg ggc ata ggc Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly 115 120 125	384
aac gga acc cag att tat gta att gat cca gaa cgg tgc cca gat tct Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser 130 135 140	432
gca gag ccc aaa tct tgt gac aaa act cac aca tgc cca cgg tgc cca Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro 145 150 155 160	480
gca cct gaa ctc ctg ggg gga cgg tca gtc ttc ctc ttc ccc cca aaa Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys 165 170 175	528
ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val 180 185 190	576
gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr 195 200 205	624
gtg gac ggc gtg gag gtg cat aat gcc aag aca aag cgg cgg gag gag Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu 210 215 220	672
cag tac aac age acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His 225 230 235 240	720
cag gac tgg ctg aat gcc aag gay tac aag tgc aag gtc tcc aac aaa Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys 245 250 255	768

Sequence Listing

```

gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag      816
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
      260              265              270

ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg      864
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
      275              280              285

acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc      912
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
      290              295              300

agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac      960
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
      305              310              315              320

tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc      1008
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
      325              330              335

tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc      1056
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
      340              345              350

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag      1104
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
      355              360              365

aag agc ctc tcc ctg tct ccg ggt aaa          tga      1134
Lys Ser Leu Ser Leu Ser Pro Gly Lys
      370              375

<210> 16
<211> 377
<212> PRT
<213> Homo sapiens

<400> 16
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro

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Sequence Listing

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1           5           10          15
Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
20           25           30
Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
35           40           45
Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
50           55           60
Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
65           70           75           80
Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
85           90           95
Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
100          105          110
Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
115          120          125
Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
130          135          140
Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
145          150          155          160
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
165          170          175
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
180          185          190
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
195          200          205
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
210          215          220

```

Sequence Listing

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
225 230 235 240

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
245 250 255

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
260 265 270

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
275 280 285

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
290 295 300

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
305 310 315 320

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
325 330 335

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
340 345 350

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
355 360 365

Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375

<210> 17

<211> 1854

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1851)

Sequence Listing

<223> CD2-CD2-IgG

<220>

<221> C_region

<222> (1153)..(1654)

<223> Hinge, CH2, CH3 region

<220>

<221> misc_signal

<222> (265)..(273)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (421)..(429)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (448)..(456)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (805)..(813)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (961)..(969)

<223> N-linked glycosylation site

<220>

Sequence Listing

<221> misc_signal
<222> (988)..(996)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(27)
<223> PCR primer SEQ ID : 40 binding site

<220>
<221> primer_bind
<222> (598)..(612)
<223> PCR primer SEQ ID : 46(antisense) binding site

<220>
<221> primer_bind
<222> (612)..(630)
<223> PCR primer SEQ ID : 45 binding site

<220>
<221> primer_bind
<222> (1128)..(1158)
<223> PCR primer SEQ ID : 41(antisense) binding site

<220>
<221> primer_bind
<222> (1151)..(1173)
<223> PCR primer SEQ ID : 42 binding site

<220>
<221> primer_bind
<222> (1832)..(1854)
<223> PCR primer SEQ ID : 28(antisense) binding site

Sequence Listing

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<220>
<221>     sig_peptide
<222>     (1)..(72)
<223>     signal peptide

<400>      17
atg agc ttt cca tgt aaa ttt gta gcc agc ttc ctt ctg att ttc aat      48
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
   1         5         10        15

gtt tct tcc aaa ggt gca gtc tcc aaa gag att acg aat gcc ttg gaa      96
Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
   20        25        30

acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac att oct agt ttt     144
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
   35        40        45

caa atg agt gat gat att gac gat ata aaa tgg gaa aaa act tca gac     192
Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
   50        55        60

aag aaa aag att gca caa ttc aga aaa gag aaa gag act ttc aag gaa     240
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
   65        70        75        80

aaa gat aca tat aag cta ttt aaa aat gga act ctg aaa att aag cat     288
Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
   85        90        95

ctg aag acc gat gat cag gat atc tac aag gta tca ata tat gat aca     336
Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
  100        105        110

aaa gga aaa aat gty ttg gaa aaa ata ttt gat ttg aag att caa gag     384
Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
  115        120        125

```

Sequence Listing

agg gtc tca aaa cca aag atc tcc tgg act tgt atc aac aca acc ctg Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu 130 135 140	432
acc tgt gag gta atg aat gga act gac ccc gaa tta aac ctg tat caa Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln 145 150 155 160	480
gat ggg aaa cat cta aaa ctt tct cag agg gtc atc aca cac aag tgg Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp 165 170 175	528
acc acc agc ctg agt gca aaa ttc aag tgc aca gca ggg aac aaa gtc Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val 180 185 190	576
agc aag gaa tcc agt gtc gag cct gtc agc tgt cct aaa gag att acg Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Lys Glu Ile Thr 195 200 205	624
aat gcc ttg gaa acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp 210 215 220	672
att cct agt ttt caa atg agt gat gat att gac gat ata aaa tgg gaa Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu 225 230 235 240	720
aaa act tca gac aag aaa aag att gca caa ttc aga aaa gag aaa gag Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu 245 250 255	768
act ttc aag gaa aaa gat aca tat aag cta ttt aaa aat gga act ctg Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu 260 265 270	816
aaa att aag cat ctg aag acc gat gat cag gat atc tac aag gta tca Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser 275 280 285	864

Sequence Listing

ata tat gat aca aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg	912
Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu	
290 295 300	
sag att caa gag agg gtc tca aaa cca aag atc tcc tgg act tgt atc	960
Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile	
305 310 315 320	
aac sca acc ctg acc tgt gag gta atg aat gga act gac ccc gaa tta	1008
Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu	
325 330 335	
aac ctg tat caa gat ggg aaa cat cta aaa ctt tct cag agg gtc atc	1056
Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile	
340 345 350	
aca cac aag tgg acc acc agc ctg agt gca aaa ttc aag tgc aca gca	1104
Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala	
355 360 365	
ggg aac aaa gtc agc aag gaa tcc agt gtc gag cct gtc agc tgt cct	1152
Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro	
370 375 380	
gca gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca	1200
Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro	
385 390 395 400	
gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa	1248
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys	
405 410 415	
ccc aag gac acc ctc atg atc tcc ccg acc cct gag gtc aca tgc gtg	1296
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val	
420 425 430	
gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac	1344
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr	
435 440 445	

Sequence Listing

gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu 450 455 460	1392
cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc tgt cac Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His 465 470 475 480	1440
cag gac tgg ctg aat gcc aag gag tac aag tgc aag gtc tcc aac aaa Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys 485 490 495	1498
gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Cys Glu 500 505 510	1536
ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu 515 520 525	1584
acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro 530 535 540	1632
agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn 545 550 555 560	1680
tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu 565 570 575	1728
tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val 580 585 590	1776
ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln 595 600 605	1824

Sequence Listing

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aag agc ctc tcc ctg tct cgg ggt aaa          tga          1854
Lys Ser Leu Ser Leu Ser Pro Gly Lys
    610          615

<210> 18
<211> 617
<212> PRT
<213> Homo sapiens

<400> 18
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
    1          5          10          15

Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
    20          25          30

Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
    35          40          45

Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
    50          55          60

Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
    65          70          75          80

Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
    85          90          95

Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
    100          105          110

Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
    115          120          125

Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
    130          135          140

Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
    145          150          155          160

```

Sequence Listing

```

Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
      165              170              175

Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
      180              185              190

Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Lys Glu Ile Thr
      195              200              205

Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp
      210              215              220

Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu
      225              230              235              240

Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu
      245              250              255

Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu
      260              265              270

Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser
      275              280              285

Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu
      290              295              300

Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile
      305              310              315              320

Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu
      325              330              335

Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile
      340              345              350

Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala
      355              360              365

```

Sequence Listing

Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro
370 375 380

Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
385 390 395 400

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
405 410 415

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
420 425 430

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
435 440 445

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
450 455 460

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His
465 470 475 480

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
485 490 495

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
500 505 510

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
515 520 525

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
530 535 540

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
545 550 555 560

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
565 570 575

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val

Sequence Listing

580 585 590

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln

595 600 605

Lys Ser Leu Ser Leu Ser Pro Gly Lys

610 615

<210> 19

<211> 1509

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1506)

<223> CTLA4-CTLA4-IgG

<220>

<221> C_region

<222> (808)..(1509)

<223> Hinge, CH2, CH3 region

<220>

<221> misc_signal

<222> (289)..(297)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (385)..(393)

<223> N-linked glycosylation site

<220>

<221> misc_signal

Sequence Listing

<222> (664)..(672)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (760)..(768)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 43 binding site

<220>
<221> primer_bind
<222> (418)..(431)
<223> PCR primer SEQ ID : 46(antisense) binding site

<220>
<221> primer_bind
<222> (432)..(453)
<223> PCR primer SEQ ID : 47 binding site

<220>
<221> primer_bind
<222> (784)..(813)
<223> PCR primer SEQ ID : 44(antisense) binding site

<220>
<221> primer_bind
<222> (805)..(826)
<223> PCR primer SEQ ID : 42 binding site

Sequence Listing

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<220>
<221> primer_bind
<222> (1486)..(1509)
<223> PCR primer SEQ ID : 28 (antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(63)
<223> signal peptide

<400> 19
atg agg acc tgg ccc tgc act ctc ctg ttt ttt ctt ctc ttc atc cct      48
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
      1          5          10          15

gtc ttc tgc aaa gca atg cac gtg gcc cag cct gct gtg gta ctg gcc      96
Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
      20          25          30

agc agc cga gcc atc gcc agc ttt gtg tgt gag tat gca tct cca gcc      144
Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
      35          40          45

aaa gcc act gag gtc cgg gtg aca gtg ctt cgg cag got gac agc cag      192
Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
      50          55          60

gtg act gaa gtc tgt gcg gca acc tac atg atg ggg aat gag ttg acc      240
Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
      65          70          75          80

ttc cta gat gat tcc atc tgc acg gcc acc tcc agt gga aat caa gtg      288
Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
      85          90          95

aac ctc act atc caa gga ctg agg gcc atg gac acg gga ctc tac atc      336
Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
      100          105          110

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Sequence Listing

tgc aag gtg gag ctc atg tac cca cgg cca tac tac ctg ggc ata ggc	364
Cys Lys Val Glu Leu Met Tyr Pro Pro Tyr Tyr Leu Gly Ile Gly	
115 120 125	
aac gga acc cag att tat gta att gat cca gaa cgg tgc cca gat tgg	432
Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser	
130 135 140	
gat aac atg cac gtg gcc cag cct gct gtg gta ctg gcc agc agc cga	480
Asp Asn Met His Val Ala Gln Pro Ala Val Val Leu Ala Ser Ser Arg	
145 150 155 160	
ggc atc gcc agc ttt gtg tgt gag tat gca tct cca ggc aaa gcc act	528
Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly Lys Ala Thr	
165 170 175	
gag gtc cgg gtg aca gtg ctt cgg cag gct gac agc cag gtg act gaa	576
Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln Val Thr Glu	
180 185 190	
gtc tgt ggg gca acc tac atg atg ggg aat gag ttg acc ttc cta gat	624
Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr Phe Leu Asp	
195 200 205	
gat tcc atc tgc acg ggc acc tcc agt gga aat caa gtg aac ctc act	672
Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val Asn Leu Thr	
210 215 220	
atc caa gga ctg agg gcc atg gac acg gga ctc tac atc tgc aag gtg	720
Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile Cys Lys Val	
225 230 235 240	
gag ctc atg tac cca cgg cca tac tac ctg ggc ata ggc aac gga acc	768
Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly Asn Gly Thr	
245 250 255	
cag att tat gta att gat cca gaa cgg tgc cca gat tct gca gag ccc	816
Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser Ala Glu Pro	
260 265 270	

Sequence Listing

aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu	864
275 280 285	
ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp	912
290 295 300	
acc ctc atg atc tcc cgg acc cct gag gtc sca tgc gtg gtg gtg gac Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp	960
305 310 315 320	
gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Glu	1008
325 330 335	
gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn	1056
340 345 350	
agc acg tac cgg gtg gtc agc gtc ctc acc gtc tgt cac cag gac tgg Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His Gln Asp Trp	1104
355 360 365	
ctg aat gcc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro	1152
370 375 380	
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu	1200
385 390 395 400	
cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn	1248
405 410 415	
cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile	1296
420 425 430	

Sequence Listing

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gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc      1344
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
      435              440              445

acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag      1392
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
      450              455              460

ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc      1440
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
      465              470              475              480

tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc      1488
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
      485              490              495

tcc ctg tct ccg ggt aaa      tga      1509
Ser Leu Ser Pro Gly Lys
      500

<210> 20
<211> 502
<212> PRT
<213> Homo sapiens

<400> 20
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
  1              5              10              15
Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
      20              25              30
Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
      35              40              45
Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
      50              55              60

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Sequence Listing

Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
 65 70 75 80

Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
 85 90 95

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
 100 105 110

Cys Lys Val Glu Leu Met Tyr Pro Pro Tyr Tyr Leu Gly Ile Gly
 115 120 125

Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
 130 135 140

Asp Asn Met His Val Ala Gln Pro Ala Val Val Leu Ala Ser Ser Arg
 145 150 155 160

Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly Lys Ala Thr
 165 170 175

Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln Val Thr Glu
 180 185 190

Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr Phe Leu Asp
 195 200 205

Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val Asn Leu Thr
 210 215 220

Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile Cys Lys Val
 225 230 235 240

Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly Asn Gly Thr
 245 250 255

Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser Ala Glu Pro
 260 265 270

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu

Sequence Listing

275	280	285
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp		
290	295	300
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp		
305	310	315
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly		
325	330	335
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn		
340	345	350
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His Gln Asp Trp		
355	360	365
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro		
370	375	380
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu		
385	390	395
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn		
405	410	415
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile		
420	425	430
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr		
435	440	445
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys		
450	455	460
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys		
465	470	475
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu		
485	490	495

Sequence Listing

Ser Leu Ser Pro Gly Lys

500

<210> 21

<211> 1854

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1851)

<223> mgCD2-CD2-IgG

<220>

<221> C_region

<222> (1153)..(1854)

<223> Hinge, CH2, CH3 region

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<221> misc_signal

<222> (265)..(273)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (421)..(429)

<223> N-linked glycosylation site

<220>

<221> misc_signal

<222> (448)..(456)

<223> N-linked glycosylation site

Sequence Listing

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<222> (598)..(606)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (616)..(624)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (805)..(813)
<223> N-linked glycosylation site

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<221> misc_signal
<222> (961)..(969)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (988)..(996)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(27)
<223> PCR primer SEQ ID : 40 binding site

<220>
<221> primer_bind
<222> (588)..(630)
<223> PCR primer SEQ ID : 50(antisense) binding site

Sequence Listing

<220>
 <221> primer_bind
 <222> (588)..(630)
 <223> PCR primer SEQ ID : 49 binding site

<220>
 <221> primer_bind
 <222> (1128)..(1158)
 <223> PCR primer SEQ ID : 41(antisense) binding site

<220>
 <221> primer_bind
 <222> (1151)..(1173)
 <223> PCR primer SEQ ID : 42 binding site

<220>
 <221> primer_bind
 <222> (1832)..(1854)
 <223> PCR primer SEQ ID : 28(antisense) binding site

<220>
 <221> sig_peptide
 <222> (1)..(72)
 <223> signal peptide

<400> 21 48
 atg agc ttt cca tgt aaa ttt gta gcc agc ttc ctt ctg att ttc aat
 Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
 1 5 10 15

gtt tot too aaa ggt gca gtc tcc aaa gag att acg aat gcc ttg gaa 96
 Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
 20 25 30

Sequence Listing

acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac att cct agt ttt Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe 35 40 45	144
caa atg agt gat gat att gac gat ata aaa tgg gaa aaa act tca gac Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp 50 55 60	192
aag aaa aag att gca caa ttc aga aaa gag aaa gag act ttc aag gaa Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu 65 70 75 80	240
aaa gat aca tat aag cta ttt aaa aat gga act ctg aaa att aag cat Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His 85 90 95	288
ctg aag acc gat gat cag gat atc tac aag gta tca ata tat gat aca Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr 100 105 110	336
aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg aag att caa gag Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu 115 120 125	384
agg gtc tca aaa cca aag atc tcc tgg act tgt atc aac aca acc ctg Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu 130 135 140	432
acc tgt gag gta atg aat gga act gac ccc gaa tta aac ctg tat caa Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln 145 150 155 160	480
gat ggg aaa cat cta aaa ctt tct cag agg gtc atc aca cac aag tgg Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp 165 170 175	528
acc acc agc ctg agt gca aaa ttc aag tgc aca gca ggg aac aaa gtc Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val 180 185 190	576

Sequence Listing

agc aag gaa tcc agt gtc gag aat gtc agc tgt cct aaa aat att acg Ser Lys Glu Ser Ser Val Glu Asn Val Ser Cys Pro Lys Asn Ile Thr 195 200 205	624
aat gcc ttg gaa acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp 210 215 220	672
att cct agt ttt caa atg agt gat gat att gac gat ata aaa tgg gaa Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Ile Lys Trp Glu 225 230 235 240	720
aaa act tca gac aag aaa aag att gca caa ttc aga aaa gag aaa gag Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg Lys Lys Glu Lys Glu 245 250 255	768
act ttc aag gaa aaa gat aca tat aag cta ttt aaa aat gga act ctg Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu 260, 265 270	816
aaa att aag cat ctg aag acc gat gat cag gat atc tac aag gta tca Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser 275 280 285	864
ata tat gat aca aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu 290 295 300	912
aag att caa gag agg gtc tca aaa cca aag atc tcc tgg act tgt atc Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile 305 310 315 320	960
aac aca acc ctg acc tgt gag gta atg aat gga act gac ccc gaa tta Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu 325 330 335	1008
aac ctg tat caa gat ggg aaa cat cta aaa ctt tct cag agg gtc atc Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile 340 345 350	1056

Sequence Listing

aca cac aag tgg acc acc agc ctg agt gca aaa ttc aag tgc aca gca Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala	1104
355 360 365	
ggg aac aaa gtc agc aag gaa tcc agt gtc gag cct gtc agc tgt cct Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro	1152
370 375 380	
gca gag ccc aaa tct tgt gac aaa act cac aca tgc cca cgg tgc cca Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro	1200
385 390 395 400	
gca cct gaa ctc ctg ggg gga cgg tca gtc ttc ctc ttc ccc cca aaa Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys	1248
405 410 415	
ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val	1296
420 425 430	
gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr	1344
435 440 445	
gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu	1392
450 455 460	
cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc tgt cac Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His	1440
465 470 475 480	
cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys	1488
485 490 495	
gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln	1536
500 505 510	

Sequence Listing

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ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg      1584
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
      515              520              525

acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc      1632
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
      530              535              540

agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac      1680
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
      545              550              555              560

tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc      1728
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
      565              570              575

tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac ctg      1776
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
      580              585              590

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag      1824
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
      595              600              605

aag agc ctc tcc ctg tct ccg ggt aaa          tga      1854
Lys Ser Leu Ser Leu Ser Pro Gly Lys
      610              615

<210> 22
<211> 617
<212> PRT
<213> Homo sapiens

<400> 22
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
      1              5              10              15

Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu

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Sequence Listing

20	25	30
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe		
35	40	45
Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp		
50	55	60
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu		
65	70	75
Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His		
85	90	95
Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr		
100	105	110
Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu		
115	120	125
Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu		
130	135	140
Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln		
145	150	155
Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp		
165	170	175
Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val		
180	185	190
Ser Lys Glu Ser Ser Val Glu Asn Val Ser Cys Pro Lys Asn Ile Thr		
195	200	205
Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp		
210	215	220
Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu		
225	230	235
		240

Sequence Listing

Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu
245 250 255

Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu
260 265 270

Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser
275 280 285

Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu
290 295 300

Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile
305 310 315 320

Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu
325 330 335

Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile
340 345 350

Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala
355 360 365

Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro
370 375 380

Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
385 390 395 400

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
405 410 415

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
420 425 430

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
435 440 445

Sequence Listing

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
450 455 460

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His
465 470 475 480

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
485 490 495

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
500 505 510

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
515 520 525

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
530 535 540

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
545 550 555 560

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
565 570 575

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
580 585 590

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
595 600 605

Lys Ser Leu Ser Leu Ser Pro Gly Lys
610 615

<210> 23
<211> 1509
<212> DNA
<213> Homo sapiens

<220>

Sequence Listing

<221> CDS
<222> (1)..(1506)
<223> mgCTLA4-CTLA4-IgG

<220>
<221> C_region
<222> (808)..(1509)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (289)..(297)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (385)..(393)
<223> N-linked glycosylation site

<220>
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<222> (403)..(411)
<223> N-linked glycosylation site

<220>
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<222> (424)..(432)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (439)..(447)
<223> N-linked glycosylation site

Sequence Listing

<220>
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<222> (664)..(672)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (760)..(768)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 43 binding site

<220>
<221> primer_bind
<222> (394)..(456)
<223> PCR primer SEQ ID : 52(antisense) binding site

<220>
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<222> (397)..(460)
<223> PCR primer SEQ ID : 51 binding site

<220>
<221> primer_bind
<222> (784)..(813)
<223> PCR primer SEQ ID : 44(antisense) binding site

<220>
<221> primer_bind
<222> (805)..(826)

Sequence Listing

<233> PCR primer SEQ ID : 42 binding site

<220>

<221> primer_bind

<222> (1486)..(1509)

<223> PCR primer SEQ ID : 28(antisense) binding site

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<221> sig_peptide

<222> (1)..(63)

<223> signal peptide

<400> 23

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Met	Arg	Thr	Trp	Pro	Cys	Thr	Leu	Leu	Phe	Phe	Leu	Leu	Phe	Ile	Pro	
1			5					10					15			

gtc	ttc	tgc	aaa	gca	atg	cac	gtg	gcc	cag	cct	gct	gtg	gta	ctg	gcc	96
Val	Phe	Cys	Lys	Ala	Met	His	Val	Ala	Gln	Pro	Ala	Val	Val	Leu	Ala	
			20				25						30			

agc	agc	cga	ggc	atc	gcc	agc	ttt	gtg	tgt	gag	tat	gca	tct	cca	ggc	144
Ser	Ser	Arg	Gly	Ile	Ala	Ser	Phe	Val	Cys	Glu	Tyr	Ala	Ser	Pro	Gly	
			35				40					45				

aaa	gcc	act	gag	gtc	cgg	gtg	aca	gtg	ctt	cgg	cag	gct	gac	agc	cag	192
Lys	Ala	Thr	Glu	Val	Arg	Val	Thr	Val	Leu	Arg	Gln	Ala	Asp	Ser	Gln	
			50				55					60				

gtg	act	gaa	gtc	tgt	gcg	gca	acc	tac	atg	atg	ggg	aat	gag	ttg	acc	240
Val	Thr	Glu	Val	Cys	Ala	Ala	Thr	Tyr	Met	Met	Gly	Asn	Glu	Leu	Thr	
			65				70				75			80		

ttc	cta	gat	gat	tcc	atc	tgc	acg	ggc	acc	tcc	agt	gga	aat	caa	gtg	288
Phe	Leu	Asp	Asp	Ser	Ile	Cys	Thr	Gly	Thr	Ser	Ser	Gly	Asn	Gln	Val	
							85				90			95		

Sequence Listing

aac ctc act atc caa gga ctg agg gcc atg gac acg gga ctc tac atc Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile 100 105 110	336
tgc aag gtg gag ctc atg tac cca ccg cca tac tac ctg ggc ata ggc Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly 115 120 125	384
aac gga acc cag att tat gta aat gat aca gaa cgg tgc aat gat tgg Asn Gly Thr Gln Ile Tyr Val Asn Asp Thr Glu Pro Cys Asn Asp Ser 130 135 140	432
gat aac aat cac acg gcc cag cct gct gtg gta ctg gcc agc agc cga Asp Asn Asn His Thr Ala Gln Pro Ala Val Val Leu Ala Ser Ser Arg 145 150 155 160	480
ggc atc gcc agc ttt gtg tgt gag tat gca tct cca ggc aaa gcc act Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly Lys Ala Thr 165 170 175	528
gag gtc cgg gtg aca gtg ctt cgg cag gct gac agc cag gtg act gaa Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln Val Thr Glu 180 185 190	576
gtc tgt ggc gca acc tac atg atg ggg aat gag ttg acc ttc cta gat Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr Phe Leu Asp 195 200 205	624
gat tcc atc tgc acg ggc acc tcc agt gga aat caa gtg aac ctc act Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val Asn Leu Thr 210 215 220	672
atc caa gga ctg agg gcc atg gac acg gga ctc tac atc tgc aag gtg Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile Cys Lys Val 225 230 235 240	720
gag ctc atg tac cca cgg cca tac tac ctg ggc ata ggc aac gga acc Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly Asn Gly Thr 245 250 255	768

Sequence Listing

cag att tat gta att gat cca gaa cgg tgc cca gat tct gca gag ccc Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser Ala Glu Pro 260 265 270	816
aaa tct tgt gac aaa act cac aca tgc cca cgg tgc cca gca cct gaa Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu 275 280 285	864
ctc ctg ggg gga cgg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp 290 295 300	912
acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp 305 310 315 320	960
gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly 325 330 335	1008
gtg gag gtg cat aat gcc aag aca aag cgg cgg gag gag cag tac aac Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn 340 345 350	1056
agc acg tac cgg gtg gtc agc gtc ctc acc gtc tgt cac cag gac tgg Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His Gln Asp Trp 355 360 365	1104
ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro 370 375 380	1152
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu 385 390 395 400	1200
cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn 405 410 415	1248

Sequence Listing

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cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc      1296
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
      420              425              430

gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc      1344
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
      435              440              445

acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag      1392
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys
      450              455              460

ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc      1440
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
      465              470              475              480

tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc      1488
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
      485              490              495

tcc ctg tct ccg ggt aaa      tga      1509
Ser Leu Ser Pro Gly Lys
      500

<210> 24
<211> 502
<212> PRT
<213> Homo sapiens

<400> 24
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
  1              5              10              15

Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
      20              25              30

Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
      35              40              45

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Sequence Listing

Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
 50 55 60
 Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
 65 70 75 80
 Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
 85 90 95
 Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
 100 105 110
 Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
 115 120 125
 Asn Gly Thr Gln Ile Tyr Val Asn Asp Thr Glu Pro Cys Asn Asp Ser
 130 135 140
 Asp Asn Asn His Thr Ala Gln Pro Ala Val Val Leu Ala Ser Ser Arg
 145 150 155 160
 Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly Lys Ala Thr
 165 170 175
 Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln Val Thr Glu
 180 185 190
 Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr Phe Leu Asp
 195 200 205
 Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val Asn Leu Thr
 210 215 220
 Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile Cys Lys Val
 225 230 235 240
 Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly Asn Gly Thr
 245 250 255
 Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser Ala Glu Pro

Sequence Listing

260	265	270
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu		
275	280	285
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp		
290	295	300
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp		
305	310	315
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly		
325	330	335
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn		
340	345	350
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His Gln Asp Trp		
355	360	365
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro		
370	375	380
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu		
385	390	395
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn		
405	410	415
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile		
420	425	430
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr		
435	440	445
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys		
450	455	460
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys		
465	470	480

Sequence Listing

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
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Ser Leu Ser Pro Gly Lys
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<210> 25
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> PCR primer, oligonucleotide TNFR1-EDF-EcoRI

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<210> 26
 <211> 37
 <212> DNA
 <213> Artificial Sequence

<220>
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<400> 26
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<210> 27
 <211> 37
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 <213> Artificial Sequence

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Sequence Listing

<223> PCR primer, oligonucleotide IgG1-T1F

<400> 27

gaggactcag gcaccacagc agagcccaa tcttgtg

37

<210> 28

<211> 34

<212> DNA

<213> Artificial Sequence

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gctctagagc tcatttaccc ggagacaggg agag

34

<210> 29

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

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<400> 29

ccggaattcc gggcaccat ggagcccgtc gcc

33

<210> 30

<211> 37

<212> DNA

<213> Artificial Sequence

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Sequence Listing

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<220>
<223> PCR primer, oligonucleotide IgG-T2F

<400> 31
gaaggagaca ctggcgagcg agagcccaaa tcttctg 37

<210> 32
<211> 37
<212> DNA
<213> Artificial Sequence

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<223> PCR primer, oligonucleotide TNFR1-CF-BamHI

<400> 32
cgcggtacgg ggaacatttc actggtccct cacctag 37

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Sequence Listing

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<210> 34
<211> 36
<212> DNA
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<220>
<223> PCR primer, oligonucleotide TNFR2-CF-BamHI

<400> 34
cgcggtatcga acgcaactac accctacgcc ccggag 36

<210> 35
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR2-NR-BamHI

<400> 35
cgcggtatcgg ctcccttcag ctggggggct g 31

<210> 36
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide mgTNFR1-TNFR1-IgG-F

Sequence Listing

<400> 36
 aaaagcaacg agaccaacaa gacctgccta cacaacgggt ccaggagaa gaacgatagt 60
 gtg 63

 <210> 37
 <211> 62
 <212> DNA
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 <220>
 <223> PCR primer, oligonucleotide mgTNFR1-TNFR1-IgG-R

 <400> 37
 ctcacctgac ccgttgtgta ggcagggtctt gttggtctcg ttgcttttct tacagttact 60
 ac 62

 <210> 38
 <211> 45
 <212> DNA
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 <220>
 <223> PCR primer, oligonucleotide mgTNFR2-TNFR2-IgG-F

 <400> 38
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 <210> 39
 <211> 42
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<210> 40

<211> 36

<212> DNA

<213> Artificial Sequence

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<223> PCR primer, oligonucleotide CD2F-EcoRI

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cgggaattca tgagctttcc atgtaaattt gtacc

36

<210> 41

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer, oligonucleotide CD2R-PstI

<400> 41

ctctgcagga cagctgacag gctcgacact

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<210> 42

<211> 25

<212> DNA

<213> Artificial Sequence

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<223> PCR primer, oligonucleotide IgG-F-PstI

Sequence Listing

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<210> 43
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<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CTL4MF-EcoRI

<400> 43
caggaaatca tgaggacctg gcc 24

<210> 44
<211> 30
<212> DNA
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<220>
<223> PCR primer, oligonucleotide CTL4MR-PstI

<400> 44
ctctgcagaa tctgggcacg gtccaggatc 30

<210> 45
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CD2-NT-F

Sequence Listing

<400> 45
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<210> 46
<211> 18
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<220>
<223> PCR primer, oligonucleotide CD2-CT-R

<400> 46
tgcaggacag ctgacagg 18

<210> 47
<211> 23
<212> DNA
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<223> PCR primer, oligonucleotide CTLA4-NT-F

<400> 47
ggataatcat gcacgtggcc cag 23

<210> 48
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CTLA4-CT-R

Sequence Listing

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<210> 49

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer, oligonucleotide mgCD2-CD2-IgG-F

<400> 49

cagtgtag aatgtcagct gtctataaaa tattacgaat gcc

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<210> 50

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer, oligonucleotide mgCD2-CD2-IgG-R

<400> 50

ggcattogta atatttttag gacagctgac attctcgaca ctg

43

<210> 51

<211> 64

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer, oligonucleotide mgCTLA4-CTLA4-IgG-F

<400> 51

Sequence Listing

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gctg 64

<210> 52

<211> 63

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer, oligonucleotide mgCTLA4-CTLA4-IgG-R

<400> 52

agcctgggct gctgtgttgt tatccgaatc attgcacggt totgtatcgt ttacataaat 60

ctg 63

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR02/01427

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07K 16/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C07K 16/46, C07K 19/00, C12N 15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,073,627 A (Immunex Corporation) 17 DECEMBER 1991 see the whole document	1
X, P ----- Y, P	EP1148065 A1 (ROSE-JOHN, STEFAN) 24 OCTOBER 2001 see column3, lines 20-40, claims	1 --- 2-5, 7-10, 12, 14, 15
Y	EP0464533 A1 (HOECHST AKTIENGESELLSCHAFT) 8 JANUARY 1992 see claims	2-5, 7-10, 12, 14, 15
Y	US 5861151 A (BRISTOL-MYERS SQUIBB CO.) 19 JANUARY 1999 see column7, lines 40-45, Fig.1	2-5, 7-10, 12, 14, 15
A	US 5349053 A (PROTEIN DESIGN LABS, INC) 20 SEPTEMBER 1994 see the whole document	1-35
A	US 5428130 A (GENENTECH, INC) 27 JUNE 1995 see the whole document	1-35
A	US 6165476 A (BETH ISRAEL DEACONESS MEDICAL) 26 DECEMBER 2000 see the whole document	1-35

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 DECEMBER 2002 (11.12.2002)

Date of mailing of the international search report

12 DECEMBER 2002 (12.12.2002)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
920 Dusan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

HAN, Hyun Sook

Telephone No. 82-42-481-5596



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR02/01427

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5073627 A	17.12.91	AU 6424090 A1 EP 0489116 B1 WO9102754 A1	03.04.91 06.04.94 07.03.91
EP1148065 A1	24.10.01	NONE	
EP 0464533 A1	08.01.92	JP 5247094 A2 KR 0249572 B1 US 20010053539 A1	24.09.93 15.03.00 20.12.01
US 5861151 A1	19.01.99	AU 03327293 A1 EP 0619843 A1 WO 9313210 A1	28.07.93 19.10.94 19.01.99
US 5349053 A1	20.09.94	NONE	
US 5428130 A1	27.06.95	EP 1029870 A2 JP 5503009 T2 WO 9108298 A2	23.08.00 27.03.93 13.06.91
US 6165476 A1	26.12.00	AU 8392198 A1 JP 2001510682 T2 WO 9902711 A3	08.02.99 07.08.01 02.09.99

Form PCT/ISA/210 (patent family annex) (July 1998)